COMPOSITIONS AND METHODS RELATED TO CANINE IgG AND CANINE IL-13 RECEPTORS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application Serial No. 60/195,874, filed April 7, 2000, entitled "Canine Immunoglobulin G Molecules and Related Methods"; and U.S. Provisional Application Serial No. 60/195,659, filed April 7, 2000, entitled "Canine IL-13 Receptors, Proteins, Nucleic Acids and Uses Thereof."

FIELD OF THE INVENTION

The present invention relates to novel canine proteins, and more particularly to canine IgG and canine interleuken-13 receptor proteins, fusion proteins, nucleic acid molecules encoding such proteins and methods of making and using the same.

BACKGROUND OF THE INVENTION

Regulation of immune and inflammatory responses in animals is important in disease management. Immune responses can be regulated by modifying the activity of immunoregulatory molecules and immune cells. Such immunoregulatory molecules include, for example, cytokines, chemokines as well as soluble and membrane-bound immunoglobulin molecules.

One type of immunoregulatory molecule is immunoglobulin, a class of which is immunoglobulin G (IgG). The DNA and amino acid sequences of IgG molecules from several species have been reported. Peptides derived from known IgG sequences have been used to generate antibodies which alter IgG function. In humans and mice, IgGs have been fairly well characterized. In general, IgGs have been characterized by function and not DNA similarity since DNA similarity is not a reliable indicator of function.

Another type of immunoregulatory molecule is interleuken-13 (IL-13). Interleukin-13 is a cytokine produced by activated type 2 helper cells (Th2 cells). IL-13 promotes growth and differentiation of B cells, and IL-13 inhibits the production of inflammatory cytokines such as interleukin-1 alpha, interleukin-1 beta, interleukin-6, interleukin-8, interleukin-10 and interleukin-12 (designated as IL-1α, IL-1β, IL-6, IL-8, IL-10 and IL-12, respectively), among others.

A cDNA encoding IL-13 was first isolated from the mouse in 1989 and the human homologue was isolated in 1993. The human IL-13 gene is located on chromosome 5 q 31 which is 12 kilobases (kb) upstream of the interleukin-4 (IL-4) gene. Given the close proximity of the two genes, it is not surprising that IL-13 and IL-4 proteins share 25% sequence identity in humans and 30% sequence identity in mice. IL-13 and IL-4 are often simultaneously produced (with other cytokines) by Th2 cells. Both IL-13 and IL-4 share functional characteristics, such as inhibiting the production of inflammatory cytokines, and up-regulating the MHC class II and CD23 expression on monocytes and/or macrophages in B cells. Furthermore, IL-4 and IL-13 induce the IgE class switch in human cells *in vitro* and trigger IgG and IgM synthesis.

Both IL-13 and IL-4 have long played a role in allergy and inflammation, but until recently it has been difficult to separate the roles of these cytokines. Th2 cells are important participants in allergic conditions; as Th2 cells differentiate they produce cytokines directly or signal other allergic effector cells which induce and maintain allergic inflammatory responses. It is proposed that an allergen stimulates Th2 cells to produce IL-13 and/or IL-4, which in turn binds to IL-4R and/or IL-13R, signaling induction of IgE synthesis on B cells. Allergen-specific IgE then binds to IgE receptors on mast cells and basophils activating these cells and causing release of mediators of allergic inflammation. Induction of allergen specific Th2 differentiation represents a hallmark of allergic disease because cytokines produced by these cells induce and maintain allergic inflammatory processes. Th2 cells selectively develop and expand in the presence of IL-4. In humans, IL-13 fails to induce Th2-cell differentiation due to the lack of functional IL-13 receptors on T cells. IL-13 and IL-4 both induce IgE synthesis on B cells though IL-13 appears to be less potent in humans.

IL-4 and IL-13 receptors (referred to as IL-4R and IL-13R, respectively) share structural homology, in that both receptor complexes contain the IL-4 receptor alpha (IL-4Rα) chain which is required for signal transduction. Binding of IL-13 or IL-4 to IL-4R and IL-13R results in comparable signaling pathways. For example, if monoclonal antibodies are directed against the IL-4Rα chain (part of both the IL-4 and IL-13R complexes) IL-4 and IL-13 activity would be inhibited. Inhibition of biological activity of either of these cytokines would cause downstream regulation changes suggesting the

importance of IL-4R□ for signal transduction. IL-13R can also function as a second receptor for IL-4 in cases where the IL-4R complex is compromised.

IL-13R is expressed on many cell types such as B cells, monocytes, macrophages, basophils, eosinophils, mast cells, endothelial cells, keratinocytes and some types of tumor cells, but active receptors have not been found on human T cells or murine B cells. Generally IL-13R is present in high numbers and thought to bind IL-13 with high affinity. The human IL-13 receptor complex consists of the 140-kilodalton (kD) IL-4Rα chain, which binds IL-4 but not IL-13, and an IL-13 binding protein. cDNAs encoding two different IL-13Rα (designated as IL-13Rα1 and IL-13Rα2) proteins have been isolated from humans and mice. Human IL-13Rα1 consists of 427 amino acids and binds IL-13 with low affinity (kD~4 nanomoles/Liter) while human IL-13Rα2 is a 380amino acid protein, which binds IL-13 with high affinity (kD~50 picomoles/Liter).

Differences in IL-13 and IL-13R have been observed between species. Functional IL-13R is found on B cells in humans, while no functional IL-13R is found on B cells in mice. As such, no IgE response can be elicited from mouse B cells, so the role of IL-13 in stimulating IgE synthesis in mice remains unclear. However, it has recently been shown that IL-4 deficient mice are able to produce IgE, presumably through an IL-13 and IL-4 independent pathway. Given the differences in IL-13 activity between human and mouse, there would be no way to predict the IL-13 activity in other species, including dogs. As such there remains a need for compounds and methods to regulate an immune response in dogs through manipulation of IL-13 and IL-13R activities. The present invention satisfies this need and provides related advantages.

SUMMARY OF THE INVENTION

The present invention relates to canine IgG and canine interleuken-13 receptor (IL-13R) proteins as well as fusion proteins containing regions from canine IgG, canine IL-13R proteins or both. Also included are nucleic acid molecules encoding such proteins as well as recombinant constructs and cells containing the nucleic acid molecules, antibodies to the isolated proteins of the present invention, therapeutic compositions useful for treating canine IgG (heavy and/or light chain) and/or canine IL-13R mediated responses including, for example, vaccines, inhibitors of the proteins

and/or nucleic acid molecules, methods for treating canine IgG (heavy and/or light chain) and/or canine IL-13R –mediated responses, methods for eliciting a canine IgG (heavy and/or light chain) and/or canine IL-13R mediated immune response, and kits containing the compositions of the present invention.

In one aspect the present invention relates to different canine IgG nucleic acid molecules and the corresponding encoded amino acid sequences. In particular, the present invention relates to isolated canine IgG nucleic acid molecules having one of the following nucleic acid sequences:

(a) a nucleic acid sequence which has at least 55% identity SEQ ID NO:1, SEQ ID NO:7, or SEQ ID NO:13, wherein said identity can be determined using a DNAsis computer program and default parameters;

(b) a nucleic acid sequence which has at least 95% identity to SEQ ID NO:4, SEQ ID NO:10, or SEQ ID NO:16, wherein said identity is determined using the DNAsis computer program and default parameters;

(c) a nucleic acid sequence which encodes a first amino acid sequence which has at least 40% identity to SEQ ID NO:2, or SEQ ID NO:14, wherein said identity is determined using the DNAsis computer program and default parameters;

(d) a nucleic acid sequence which encodes a second amino acid sequence which has at least 90% identity SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11 or SEQ ID NO:17 wherein said identity is determined using the DNAsis computer program and default parameters;

(e) a nucleic acid sequence which is an allelic variant of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:13, or SEQ ID NO:16; or

(f) a nucleic acid sequence complementary to any of the above nucleic acid sequences.

The isolated nucleic acid molecules can further include the following sequences:

(a) a nucleic acid sequence which has at least 70% identity to SEQ ID NO:1, SEQ ID NO:7, or SEQ ID NO:13, wherein said identity is determined using the DNAsis computer program and default parameters;

(b) a nucleic acid sequence which encodes a third amino acid sequence which has at least 70% identity to SEQ ID NO:2, or SEQ ID NO:14, wherein said identity is determined using the DNAsis computer program and default parameters;

(c) a nucleic acid sequence which is an allelic variant of SEQ ID NO:1, SEQ ID NO:7, or SEQ ID NO:13; or

(d) a nucleic acid sequence complementary to any of the nucleic acid sequences of (a), (b) or (c).

In yet a further embodiment, the isolated canine IgG nucleic acid molecule can have the following sequences:

(a) a nucleic acid sequence comprising at least 70 contiguous nucleotides of SEQ ID NO:1, SEQ ID NO:7 or SEQ ID NO:13;

(b) a nucleic acid sequence comprising at least 350 contiguous nucleotides of SEQ ID NO:4, SEQ ID NO :10, and SEQ ID NO:16;

(c) a nucleic acid sequence comprising at least 450 contiguous nucleotides of SEQ ID NO:19;

(d) a nucleic acid sequence which encodes a first amino acid sequence comprising at least 20 contiguous residues of the sequence shown in SEQ ID NO:2, and SEQ ID NO:14;

(e) a nucleic acid sequence which encodes a second amino acid sequence comprising at least 100 contiguous residues of the sequence shown in SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, or SEQ ID NO:17;

(f) a nucleic acid sequence which encodes a third amino acid sequence comprising at least 200 contiguous residues of the sequence shown in SEQ ID NO:20; and

(g) an nucleic acid sequence complementary to any of the above nucleic acid sequence.

In yet another embodiment, the isolated canine IgG nucleic acid molecules of the present invention can contain the following nucleic acid sequences:

(a) a nucleic acid sequence comprising at least 150 contiguous nucleotides of SEQ ID NO:1, SEQ ID NO:7 or SEQ ID NO:13;

(b) a nucleic acid sequence comprising at least 500 contiguous nucleotides of SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:10 or SEQ ID NO:16;

(c) a nucleic acid sequence comprising at least 700 contiguous nucleotides of the sequence shown in SEQ ID NO:19;

(d) a nucleic acid sequence which encodes a fourth amino acid sequence comprising at least 50 contiguous residues of the sequence shown in SEQ ID NO:2 or SEQ ID NO:14;

(e) a nucleic acid sequence which encodes a fifth amino acid sequence comprising at least 200 contiguous residues of SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11 or SEQ ID NO:17;

(f) a nucleic acid sequence which encodes a sixth amino acid sequence comprising at least 300 contiguous residues of the sequence shown in SEQ ID NO:20; and

(g) a nucleic acid sequence complementary to the above nucleic acid sequences.

The isolated IgG nucleic acid molecules of the present invention can further contain the following nucleic acid sequences:

(a) a nucleic acid sequence which is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:16, and SEQ ID NO:19;

(b) a nucleic acid sequence which is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, and SEQ ID NO:20;

(c) a nucleic acid sequence complementary to any of the above nucleic acid sequences.

The present invention further provides canine IgG heavy chain proteins having an amino acid sequence encoded by any of the above-identified nucleic acid molecules or having an amino acid sequence selected from the following: SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, or SEQ ID NO:19. The invention is also directed to fusion constructs containing at least one of the above-identified nucleic acid molecules, as well as to corresponding fusion proteins. The invention additionally provides recombinant vectors and recombinant cells containing at least one of the nucleic acid molecules or fusion constructs. Isolated antibodies selective for the canine IgG proteins of the present inventon are also provided.

Methods of detecting canine IgG nucleic acid molecules are also provided and can generally be accomplished by:

(a) contacting an isolated the isolated nucleic acid molecule of the present invention with a putative IgG nucleic acid-containing composition under conditions suitable for formation of a heavy chain of canine IgG nucleic acid molecule/IgG nucleic acid complex; and

(b) detecting the presence of IgG nucleic acid by detecting the heavy chain of canine IgG nucleic acid molecule/IgG nucleic acid complex.

Also provided are kits containing any of the above isolated nucleic acid molecules, a protein encoded by the isolated nucleic acid molecules, an inhibitor of a nucleic acid molecule and/or an inhibitor of the protein encolded by the isolated nucleic acid molecules of the present invention.

The present invention also provides for canine interleukin-13 receptor (IL-13R) proteins, nucleic acid molecules encoding such proteins, antibodies raised against such proteins and/or inhibitors of such proteins or nucleic acid molecules. This aspect of the present invention particularly relates to canine interleukin-13 receptor alpha 1 (IL-13Rα1 or IL-13Ra1) and canine interleukin-13 receptor alpha 2 (IL-13Rα 2 or IL-13Ra2) proteins, nucleic acid molecules, and antibodies and inhibitors of the IL-13R proteins and nucleic acids.

In one embodiment, the present invention provides the following isolated IL-13R nucleic acid molecules:

(a) a nucleic acid molecule comprising at least 75 contiguous nucleotides identical in sequence to an at least 75 contiguous nucleotide region of SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:52 or SEQ ID NO:53;

(b) a nucleic acid molecule comprising a nucleic acid sequence that is at least 90 percent identical in sequence to SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:52 or SEQ ID NO:53, and a fragment thereof, wherein said fragment is at least 80 nucleotides in length, and wherein said percent identity can be determined by a DNAsis™ computer program with a gap penalty set at 5, the number of top diagonals set at 5, a fixed gap penalty set at 10, a k-tuple set at 2, a window size set at 10 and a floating gap penalty set at 1;

(c) isolated nucleic acid molecule encoding a protein comprising amino acid sequence SEQ ID NO: 50;

(d) isolated nucleic acid molecule selected from the group consisting of:

(i) a first nucleic acid molecule having at least 40 contiguous nucleotides identical in sequence to at least 40 contiguous nucleotide region of SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:60, SEQ ID

NO:62, SEQ ID NO:63, SEQ ID NO:64,SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68 or SEQ ID NO:70; and

(ii) a second nucleic acid molecule comprising a first nucleic acid sequence that is at least 80% identical in sequence to SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64,SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68 or SEQ ID NO:70, and a fragment thereof, wherein said fragment is at least 50 nucleotides in length, and wherein said percent identity can be determined by a DNAsis™ computer program with a gap penalty set at 5, the number of top diagonals set at 5, a fixed gap penalty set at 10, a k-tuple set at 2, a window size set at 10 and a floating gap penalty set at 10.

(e) an isolated nucleic acid molecule encoding a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:61, SEQ ID NO:66, and SEQ ID NO:69.

(f) an isolated nucleic acid molecule having a first nucleic acid sequence encoding a protein selected from the group consisting of:

(i) a protein that is at least 85 percent identical in sequence to SEQ ID NO:3, wherein said percent identity can be determined by the DNAsis™ computer program with a gap penalty set at 5, the number of top diagonals set at 5, a fixed gap penalty set at 10, a k-tuple set at 2, a window size set at 10 and a floating gap penalty set at 10; and

(ii) a protein comprising a fragment of at least 45 contiguous amino acids identical in sequence to an at least 45 contiguous amino acid sequence of the second protein;

(g) an isolated nucleic acid molecule comprising a second nucleic acid sequence encoding a protein that comprises an at least 40 contiguous amino acid region identical in sequence to an at least 40 contiguous amino acid region of SEQ ID NO:3;

(h) an isolated nucleic acid molecule contains a nucleic acid sequence encoding an IL-13Rα1 protein of at least 45 contiguous amino acids in length, wherein the nucleic acid sequence comprises an at least 135 contiguous nucleotide sequence identical in sequence to at least 135 contiguous nucleotide region of SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:52 or SEQ ID NO:53;

(i) an isolated nucleic acid molecule having a first nucleic acid sequence encoding a first protein selected from the group consisting of:

(i) a second protein comprising an amino acid sequence that is at least 70 percent identical in sequence to SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:61, SEQ ID NO:66, and SEQ ID NO:69, wherein percent identity is determined by a DNAsis™ computer program with a gap penalty set at 5, the number of top diagonals set at 5, a fixed gap penalty set at 10, a k-tuple set at 2, a window size set at 10 and a floating gap penalty set at 10;

(ii) a protein comprising a fragment of at least 40 contiguous amino acids identical in sequence to an at least 40 contiguous amino acids of the first protein;

(j) an isolated nucleic acid molecule comprising a nucleic acid sequence encoding a protein that comprises an at least 30 contiguous amino acid region identical in sequence to an at least 30 contiguous amino acid region of SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:61, SEQ ID NO:66, and SEQ ID NO:69;

(k) an isolated nucleic acid molecule of (j), wherein the protein binds to canine IL-13, as measured by its ability to inhibit IL-13-stimulated TF-1 cell proliferation;

(l) an isolated nucleic acid molecule of (j), wherein the isolated nucleic acid molecule contains a nucleic acid sequence that encodes an IL-13Rα2 protein of at least 40 amino acids in length, wherein said nucleic acid sequence comprises an at least 120 contiguous nucleotide sequence identical in sequence to an at least 120 contiguous nucleotide region of SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64,SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68 or SEQ ID NO:70, wherein said isolated nucleic acid molecule does not hybridize under conditions comprising hybridization at 65°C in 0.1 X SSC followed by washing at 65°C in 0.1 X SSC with the third nucleic acid sequence selected from the group consisting of SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97 and SEQ ID NO:98; and

(m) an isolated nucleic acid molecule complementary of any of the above isolated nucleic acid molecules.

In another embodiment of the invention, proteins encoded by the above nucleic acid molecules are also provided. Such proteins include the following:

(a) a protein comprising an at least 40 contiguous amino acid region identical in sequence to an at least 40 contiguous amino acid region of SEQ ID NO:50;

(b) a protein comprising an amino acid sequence that is at least 85 percent identical in sequence to amino acid sequence SEQ ID NO:50 and a fragment thereof, wherein said fragment is at least 45 amino acids in length, wherein percent identity can be determined by a DNAsis™ computer program;

(c) a protein encoded by a nucleic acid molecule comprising an at least 120 contiguous nucleotide region identical in sequence to an at least 120 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:48, SEQ ID NO:49, and SEQ ID NO:52;

(d) a protein comprising a first amino acid sequence of at least 30 amino acids in length, wherein said first amino acid sequence has at least 30 contiguous amino acid region identical in sequence to at least 30 contiguous amino acid region of SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:61, SEQ ID NO:66, or SEQ ID NO:69;

(e) a protein comprising a third amino acid sequence that is at least 70 percent identical in sequence to SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:61, SEQ ID NO:66, or SEQ ID NO:69, and a fragment thereof, wherein said fragment is at least 40 amino acids in length, wherein percent identity is determined by a DNAsis™ computer program; and

(f) a protein encoded by a nucleic acid molecule comprising an at least 90 contiguous nucleotide region identical in sequence to an at least 90 contiguous nucleotide region of SEQ ID NO:54, SEQ ID NO:57, SEQ ID NO:60, SEQ ID NO:63, SEQ ID NO:65 or SEQ ID NO:68.

The present invention also provides chimeric nucleic acid molecules encoding a fusion protein in which the chimeric nucleic acid molecules contain a nucleic acid molecule encoding a carrier protein domain and a nucleic acid molecule encoding a canine IL-13Rα protein domain. The fusion protein can also contain a linker sequence. The carrier protein domain can be an immunoglobulin Fc region, preferably a canine immunoglobulin Fc region, and more preferably a canine immunoglobulin IgG Fc region. The canine IL-13Rα protein domain can be IL-13Rα1 or IL-13Rα2 protein domains. The chimeric nucleic acid molecule can contain the following nucleic acid sequences: SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:77, SEQ ID NO:80 and SEQ ID NO:82, as well as SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:57, SEQ ID NO:60, SEQ ID NO:63, SEQ ID NO:65, and SEQ ID NO:68.

Additionally, the chimeric nucleic acid molecules can have a nucleic acid molecule encoding the carrier protein domain on the 5' end and the nucleic acid molecule encoding the IL-13R protein domain on the 3' end. Alternatively, the domains can be reversed.

The present invention further provides fusion proteins containing a carrier protein domain and a canine IL-13Rα domain. Preferably, the fusion protein contains an amino acid sequence of SEQ ID NO:72, SEQ ID NO:75, SEQ ID NO:78, and SEQ ID NO:81, as well as SEQ ID NO:52, SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:61, SEQ ID NO:66, and SEQ ID NO:69.

Additional embodiments of the present invention include mimetopes and multimeric forms of any of the above proteins. Antibodies that selectively bind to any of the canine IL-13R proteins of the present invention are also provided, as well as recombinant vectors, fusion constructs, fusion sequences, and recombinant cells containing at least one nucleic acid molecules of the present invnetion.

The present invention also includes therapeutic compositions and kits containing such nucleic acid molecules, proteins, antibodies and/or inhibitors, as well as their use to evaluate and regulate an immune response of an animal, including naked nucleotide vaccines and recombinant cell vaccines.

Also provided are methods to produce a canine Il-13Rα proteins by culturing a recombinant cell capable of expressing the protein, as well as methods of identifying an inhibitor of canine Il-13Rα activity by contacting a canine Il-13Rα protein with a putative inhibitory compound and determining if Il-13Rα protein activity is inhibited.

DETAILED DESCRIPTION OF INVENTION

The present invention provides for isolated nucleic acid molecules which encode a canine IgG (heavy and/or light chain) protein, isolated proteins encoded by the nucleic acid molecules, recombinant constructs and cells comprising the nucleic acid molecules and/or proteins, antibodies to the isolated proteins, inhibitors of the proteins and nucleic acids, therapeutic compositions useful for treating canine IgG (heavy and/or light chain)-mediated responses (including e.g., vaccines), methods for treating canine IgG (heavy and/or light chain)-mediated responses, methods for eliciting a canine IgG (heavy and/or light chain)-mediated immune response, and kits comprising the materials provided.

According to the present invention, an isolated, or biologically pure, nucleic acid molecule or protein, is a nucleic acid molecule or protein that has been removed from its natural milieu. As such, "isolated" and/or "biologically pure" do not necessarily reflect the extent to which the nucleic acid molecule or protein has been purified. "Proteins" means any compounds that comprise amino acids, including peptides, polypeptides, fusion proteins, etc. It is further to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, a protein refers to one or more proteins or at least one protein. As such, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. It is also to be noted that the terms "comprising", "including", and "having" can be used interchangeably. Furthermore, a compound "selected from the group consisting of" refers to one or more of the compounds in the list that follows, including mixtures (e.g., combinations) of two or more of the compounds.

The present invention also comprises expression vectors and recombinant cells comprising the present nucleic acid molecules. Also provided are fusion proteins comprising canine IgG heavy chain proteins as well as fusion protein constructs encoding such fusion proteins. "Fusion protein" means a protein (including polypeptides) which are a combination of two or more protein regions or whole proteins. For example, two proteins may be fused and both functional as present in nature, or fused such that their function is altered. They may be fused by linking internal to the protein sequence of one or the other, or fused with a linker sequence. The linker sequence can be of any length, and any amino acid composition; it may contain advantageous features such as a cleavage site, phosphorylation site, glycosylation site, etc. A fusion protein can be obtained through translation of a fusion sequence. "Fusion sequence" is meant to refer to any nucleic acid sequence that is not naturally-occurring and can include: a canine sequence; a murine sequence; a equine sequence; a feline sequence; and a human sequence; a non-canine receptor sequence; a non-canine immunoglobulin sequence; and a non-canine cytokine sequence. A fusion protein can be made up of canine IgG or a portion thereof, attached, fused, joined to proteins or portions thereof (e.g. variable region of other species immunoglobulins) to "caninize"; add to other molecules for stability, or as an adjuvant.

As used herein, a canid refers to any member of the canid family (i.e. the family

Canidae), including, but not limited to, domestic dogs, and wild canids such as wolves, foxes, and coyotes. Similarly, the term canine refers to "of the family Canidae".

Canine IgG nucleic acid molecules can encode entire IgG (e.g. heavy and light chain, or portion thereof) at least Fcgamma, or fragment thereof and variable region or fragment thereof. "Fragment" is meant to refer to any subset of the referent nucleic acid molecule. Moreover, included is a light chain or a fragment thereof and may be a single chain antibody. "Antibody" as used herein includes both polyclonal and monoclonal antibodies as well as fragments thereof, such as Fv, Fab and $F(ab)_2$ fragments that are capable of binding antigen or hapten. Also included are heterospecies -e.g. Canine Fc and other species variable regions. Moreover, small, functional fragments are also included, such as, but not limited to: the hinge regions, other regions, such as regions which probide stability, complement activation, antigenic regions, or Fcgamma receptor binding regions. Each domain is included (gamma 1, gamma 2, and gamma 3), as are any uses thereof.

The present invention also provides for isolated canine interleukin-13 receptor (IL-13R), proteins, nucleic acid molecules encoding such proteins, antibodies raised against such proteins and/or inhibitors of such proteins or nucleic acid molecules. The present invention provides for isolated IL-13Rα proteins, such as canine interleukin-13 receptor alpha 1 (IL-13Rα1), canine interleukin-13 receptor alpha 2 (IL-13Rα 2), canine IL-13Rα:canine IgG fusion proteins and nucleic acid molecules, as well as antibodies raised against such proteins, and/or inhibitors of such proteins or nucleic acid molecules. Also included in the present invention is the use of these proteins, nucleic acid molecules, antibodies, and/or compounds derived therefrom as therapeutic compositions to regulate the immune response of an animal as well as in other applications, such as those disclosed below.

Also provided for in the present invention are isolated canine nucleic acid molecules that includes a canine IL-13R nucleic acid molecule. As used herein a canine IL-13R nucleic acid molecule refers to a nucleic acid molecule that includes a canine IL-13 receptor alpha chain (IL-13Rα) nucleic acid molecule that encodes a canine IL-13 receptor alpha chain protein and/or a complement thereof. Preferably, a canine IL-13Rα nucleic acid molecule of the present invention is a canine IL-13Rα1 or IL-13Rα2 nucleic

acid molecule that encodes a canine IL-13Rα1 or IL-13Rα2 protein and/or a complement thereof. As used herein, a canine nucleic acid molecule of the present invention is a nucleic acid molecule that is isolated from a canid or is derived therefrom in that it is produced using recombinant DNA technology, or by chemical synthesis. As such, a canine nucleic acid molecule includes natural forms as well as any variants thereof, such as a canine nucleic acid molecule that has been altered in a manner known to those skilled in the art, such as those methods disclosed herein. As used herein, a canine nucleic acid molecule does not refer to a mouse or human nucleic acid molecule.

Nucleic acid molecules of the present invention of known length isolated from *Canis familiaris* are denoted as follows: IL-13Rα1 is denoted as nCaIL-13Rα1_x, wherein "x" refers to the number of nucleotides in that molecule for example, nCaIL-13Rα1_{483} refers to a canine IL-13Rα1 nucleic acid molecule of 483 nucleotides; and in a similar fashion, a IL-13Rα2 nucleic acid molecule of length "x" is referred to as nCaIL-13Rα2_x. Similarly, *Canis familiaris* IL-13Rα1and IL-13Rα2 proteins of the present invention of known length isolated from are denoted PCaIL-13Rα1_x, and/or PcaIL-13Rα2_x, respectively.

One embodiment of the present invention is an isolated protein that includes a canine IL-13 receptor (IL-13R) protein. As used herein a canine IL-13R protein refers to a protein, or protein complex, that includes a canine IL-13 receptor alpha chain (IL-13Rα) protein. Preferably, a canine IL-13Rα protein of the present invention is a IL-13Rα1 protein or IL-13Rα2 protein. As used herein, a canine protein of the present invention is a protein that is isolated from a canid or is derived therefrom in that it is produced using recombinant DNA technology, or by chemical synthesis. As such, a canine protein includes natural forms as well as any variants thereof, such as a canine protein that has been altered in a manner known to those skilled in the art, such as those methods disclosed herein. As used herein, a canine protein does not refer to a mouse or human protein.

Identification of the canine IL-13R nucleic acid molecules of the present invention and particularly of the canine IL-13Rα2 nucleic acid molecules of the present invention is unexpected because initial attempts to obtain canine IL-13Rα nucleic acid

molecules using standard cDNA screening conditions were unsuccessful. While not being bound by theory, it is believed that canine mRNAs encoding IL-13Rα proteins are extremely rare (i.e. present in very low concentrations, at best, in a cell).

The following Table 1 summarizes the Sequence Listing for convenience:

Table 1

SEQ ID NO	Molecule	Description of the Sequence	Approx. Closest Identity[1]
1		DNA encoding hinge region of clone 3523	45%
2		deduced amino acid sequenced encoded by SEQ ID NO 1	34%
3		deduced complement of SEQ ID NO 1	
4		DNA encoding clone 3523	88%
5		deduced amino acid sequenced encoded by SEQ ID NO 4	86%
6		deduced complement of SEQ ID NO 4	
7		DNA encoding hinge region of clone Bly 8	51%
8		deduced amino acid sequenced encoded by SEQ ID NO 7	89%
9		deduced complement of SEQ ID NO 7	
10		DNA encoding clone Bly 8	90%
11		deduced amino acid sequenced encoded by SEQ ID NO 10	88%
12		deduced complement of SEQ ID NO 10	
13		DNA encoding hinge region of clone Bly 9	41%
14		deduced amino acid sequenced encoded by SEQ ID NO 13	16%
15		deduced complement of SEQ ID NO 13	
16		DNA encoding clone Bly 9	89%
17		deduced amino acid sequenced encoded by SEQ ID NO 16	82%
18		deduced complement of SEQ ID NO 16	
19		DNA encoding clone 4325	98%
20		deduced amino acid sequenced encoded by SEQ ID NO 19	98%
21		deduced complement of SEQ ID NO 19	

SEQ ID NO	Molecule	Description of the Sequence	Approx. Closest Identity[1]
22		DNA encoding hinge region of clone 4325	
23		deduced amino acid sequenced encoded by SEQ ID NO 22	
24		deduced complement of SEQ ID NO 2	
25		Lambda part	
26		deduced amino acid sequenced encoded by SEQ ID NO 25	
27		deduced complement of SEQ ID NO 25	
28		3523 part	
29		deduced amino acid sequenced encoded by SEQ ID NO 28	
30		deduced complement of SEQ ID NO 28	
31		4325 part	
32		deduced amino acid sequenced encoded by SEQ ID NO 31	
33		deduced complement of SEQ ID NO 31	
34		Bly 8-5 part	
35		deduced amino acid sequenced encoded by SEQ ID NO 34	
36		deduced complement of SEQ ID NO 34	
37		Bly 8-3C part	
38		deduced amino acid sequenced encoded by SEQ ID NO 37	
39		deduced complement of SEQ ID NO 37	
40		Primer C-IgG330-F	
41		Primer IgG-FWD1	
42		Primer IgG-REV1	
43		Primer IgG-REV2	
44		Primer IgG-REV3	
45		Primer IgG-REV4	
46		Primer K9 IgG 5' (F)	
47		Primer Bly 822F	
48	nCalL-13Rα1483	partial	

SEQ ID NO	Molecule	Description of the Sequence	Approx. Closest Identity[1]
49	nCaIL-13Rα11547	partial CDS? CDS 1-1215	
50	PCaIL-13Rα1405	translated sequence	
51	RC		
52	nCaIL-13Rα11215	coding for seq id no:3 CDS 1-1215	
53	RC		
54	nCaIL-13Rα2620	partial, 5' end CDS 184-620	
55	PCaIL-13Rα2145	translated sequence	
56	RC		
57	nCaIL-13Rα2878	partial, 3' end CDS 1-765	
58	PCaIL-13Rα2255	translated sequence	
59	RC		
60	nCaIL-13Rα21454	full length CDS 184-1341	
61	P CaIL-13Rα2386	full length protein+signal sequence	
62	reverse complement		
63	nCaIL-13Rα21158	coding for PCaIL-13R□2386 CDS 1-1158	
64	reverse complement		
65	nCaIL-13Rα21095	full length minus signal sequence CDS 1-1095	
66	PCaIL-13Rα2365	protein	
67	RC		
68	nCaIL-13Rα2954	extracellular portion of molecule CDS 1-954	
69	PCaIL-13Rα2318	protein for extracellular portion	
70	reverse complement		
71	nCaIL-13Rα2-Fc-3523	chimera 1686 CDS 1-1683	
72	PCaIL-13Rα2-Fc-3523	561 aa	
73	reverse complement		
74	nCaIL-13Rα2-Fc-4325	chimera 1698 CDS 1-1695	
75	PCaIL-13Rα2-Fc-4325	565 aa	

SEQ ID NO	Molecule	Description of the Sequence	Approx. Closest Identity[1]
76	reverse complement		
77	nCaIL-13Rα2-Fc-B9	chimera 1692 CDS 1-1689	
78	PCaIL-13Rα2-Fc-B9	563 aa	
79	reverse complement		
80	nCaIL-13Rα2-Fc-B8	chimera 1686 CDS 1-1683	
81	PCaIL-13Rα2-Fc-B8	561 aa	
82	reverse complement		
83	primer 13R1F1		
84	primer 13R1R1		
85	primer 13R2F1D	GARATHAARGTNAAYCCNCCNCARGAYTTYG ARAT	
86	primer 13R2F2D	TAYAARGAYGGNTTCTGAYYTNAAYAARGGN ATHGA	
87	primer 13R2R1D	CCAYTCNSWCCADATNCCRTCRTCNGCRCART ADATRTTNACYTT	
88	primer 13R2R2D	GCRTGRTCNARNCCYTCRTACCA	
89	primer 13R2F5	AGCGGATCCCTCTATGCTTTCAAATGCTGAGA TAAAAGTTAATCCTCCTCAGG	
90	primer 13R2F2	TGGACATCACCACAAGGAAATCGGG	
91	primer 13R2FcF		
92	primer 13R2FcR2		
93	primer cIgGFcF		
94	primer cIgGFcR		
95	murine IL-13R bc	No. 5,710,023 seq id no:1	
96	human IL-13R bc	No. 5,710,023 seq id no:3	
97	RC, murine IL-13R	rc of seq id no:48	
98	RC, human IL-13R	rc of seq id no:49	
99	primer 13R1F2	CTC TAC TAT TGG CAC AGC AGC CTG GGA	
100	primer 13R1R2	AGT CAG AGC AAA GGA ACA ACC AAT GTG	
101	primer 13R1F3	CCT CCC GAG GGA GCC AGC CCG	
102	primer 13R1R3	CGG GCT GGC TCC CTC GGG AGG	
103	primer 13R1F4	CAT GGT CCC CGG CGT TCT TCC	

SEQ ID NO	Molecule	Description of the Sequence	Approx. Closest Identity[1]
104	primer 13R1F5	GGT GAG AAT ACC GAC CCC ACG	

[1] These figures are the result of comparison of GenBank Accession Number E03345 with the sequences shown in the sequence listing.

Included within the scope of the present invention, with particular regard to the nucleic acid molecules of the present invention, are allelic variants, degenerate sequences and other homologues. An allelic variant of a nucleic acid molecule, including the particular SEQ ID NO's cited herein, is a gene that occurs at essentially the same locus (or loci) in the genome as the gene including the particular SEQ ID NO's cited herein, but which, due to natural variations caused by, for example, mutation or recombination, has a similar but not identical sequence. Also included in the term allelic variant are allelic variants of cDNAs derived from such genes. Because natural selection typically selects against alterations that affect function, allelic variants usually encode proteins having similar activity to that of the protein encoded by the gene to which they are being compared. Allelic variants of genes or nucleic acid molecules can also comprise alterations in the 5' or 3' untranslated regions of the gene (e.g., in regulatory control regions), or can involve alternative splicing of a nascent transcript, thereby bringing alternative exons into juxtaposition. Allelic variants are well known to those skilled in the art and would be expected to be found within a given animal, and/or among a group of two or more animals, since the respective genomes are diploid, and sexual reproduction will result in the reassortment of alleles. The present invention also includes homologues due to laboratory manipulation, such as, but not limited to, variants produced during polymerase chain reaction amplification or site directed mutagenesis. It is also well known that there is a substantial amount of redundancy in the various codons which code for specific amino acids. Therefore, this invention is also directed to those nucleic acid sequences which contain alternative codons which code for the same amino acid. Also included within the scope of this invention are homologues either in the nucleic acid sequence or the translated protein which do not substantially alter the ultimate physical properties of the expressed protein. For example, substitution of valine for leucine, arginine for lysine, or asparagine for glutamine may not cause a change in

functionality of the protein.

In particular, there are provided isolated nucleic acid molecules, wherein said nucleic acid molecules comprise a nucleic acid sequence selected from the group consisting of:

(a) a nucleic acid sequence which has at least 55% identity to a nucleic acid sequence which is selected from the group consisting of SEQ ID NO 1, SEQ ID NO 7, and SEQ ID NO 13, wherein said identity can be determined using the DNAsis computer program and default parameters,

(b) a nucleic acid sequence which has at least 95% identity to a nucleic acid sequence which is selected from the group consisting of SEQ ID NO 4, SEQ ID NO 10, and SEQ ID NO 16, wherein said identity is determined using the DNAsis computer program and default parameters,

(c) a nucleic acid sequence which encodes an amino acid sequence which has at least 40% identity to an amino acid sequence selected from the group consisting of SEQ ID NO 2, and SEQ ID NO 14, wherein said identity is determined using the DNAsis computer program and default parameters,

(d) a nucleic acid sequence which encodes an amino acid sequence which has at least 90% identity to an amino acid sequence selected from the group consisting of SEQ ID NO 5, SEQ ID NO 8, SEQ ID NO 11 and SEQ ID NO 17 wherein said identity is determined using the DNAsis computer program and default parameters,

(e) a nucleic acid sequence which is an allelic variant of a nucleic acid sequence selected from the group consisting of SEQ ID NO 1, SEQ ID NO 4, SEQ ID NO 7, SEQ ID NO 10, SEQ ID NO 13, or SEQ ID NO 16; and

(f) a nucleic acid sequence fully complementary to a nucleic acid sequence selected from the group consisting of a nucleic acid sequence of (a), a nucleic acid sequence of (b), a nucleic acid sequence of (c), a nucleic acid sequence of (d), a nucleic acid sequence of (e), and a nucleic acid sequence of (f).

Also provided are isolated nucleic acid molecules, wherein said nucleic acid molecules comprise a nucleic acid sequence selected from the group consisting of

(a) a nucleic acid sequence which has at least 70% identity to a nucleic acid sequence which is selected from the group consisting of SEQ ID NO 1, SEQ ID NO 7, and SEQ ID NO 13, wherein said identity is determined using the DNAsis computer program and default parameters,

(b) a nucleic acid sequence which encodes an amino acid sequence which has at least 70% identity to an amino acid sequence selected from the group consisting of SEQ ID NO 2, and SEQ ID NO 14, wherein said identity is determined using the DNAsis computer program and default parameters,

(c) a nucleic acid sequence which is an allelic variant of a nucleic acid sequence selected from the group consisting of SEQ ID NO 1, SEQ ID NO 7, and SEQ ID NO 13, and

(d) a nucleic acid sequence fully complementary to a nucleic acid sequence selected from the group consisting of a nucleic acid sequence of (a), a nucleic acid sequence of (b), and a nucleic acid sequence of (c).

Clone 3523 that encodes a full length canine IgG represents 95% of IgG populations in the dog spleen cell cDNA library. Clone 4325 that encodes entire constant region (C1γ, Cγ2 and Cγ3) of canine IgG and partial variable region (V_H) of the immunoglobulin consists of about 5% of IgG in the library. Two additional IgG clones, Bly8 and Bly9 that encode Cγ1, Cγ2 and Cγ3 domains of canine IgG, were identified from canine B-cell lymphoma samples and confirmed by PCR from the spleen cell cDNA library as well as cDNAs prepared from eleven dogs, although these two IgG sequences were not detected in screening the library with ^{32}P-labeled canine IgG DNA probes. The homology study indicates that the similarity among these four canine IgGs is between 72% to 85%. Over all similarity between IgG subclasses from canine, human and mouse is around 52-53%. Within the group of canine IgG, the difference among these IgG sequences is mainly in the hinge region of the molecules, although small difference also detected in Cγ2 and Cγ3. Evaluation of more than 200 canine IgG sequences from eighteen B-cell lymphoma samples and cDNAs prepared from samples

of canine T-cell lymphoma shows that amino acid sequences of hinge region is highly conserved within each canine IgG subclass in samples from different dogs. However, the hinge region is quite diverse among different canine IgG subclasses with similarity around 19-35%. These results indicate that the unique sequence in hinge region is the nature of each canine IgG subclass, and not due to the polymorphism of canine IgGs.

Identification and characterization of four DNA sequences encoding different canine IgG subclasses will have broad applications in canine immunology research as well as in canine health care practices. The applications include that: (a) the invention will enable the raising of subclass specific monoclonal antibodies; (b) the information regarding each canine subclass will help in investigating the immunological functions of the IgGs in dogs that have different immunological status; (c) the outcome of (a) and (b) may have significant impacts in clinical applications such as identifying certain diseases and monitoring immunological status of dogs during the course of immuno-therapy; and (d) furthermore, the identification of DNA and amino acid sequences encoding different canine IgG subclasses will be important in engineering canine specific therapeutic agents, for example, caninization of specific antibodies or constructing immunoadhesins for certain diseases and immunological disorders.

Also provided for in the present invention are canine IL-13Rα1 nucleic acid molecules that includes one or more of the following nucleic acid sequences:

(a) the nucleic acid sequence SEQ ID NO:1 and/or

(b) SEQ ID NO:49, and/or

(c) a complements of these nucleic acid sequences, i.e. SEQ ID NO:51 and/or SEQ ID NO:53, respectively.

These nucleic acid sequences are further described herein. For example, nucleic acid sequence SEQ ID NO:48 represents the deduced nucleic acid sequence of a coding strand that encodes a partial (i.e. non-full length) IL-13Rα1 protein. SEQ ID NO:49 represents the deduced sequence of the coding strand of a canine IL-13Rα1 nucleic acid molecule nCaIL-13Rα1$_{1547}$, the cloning of which is disclosed in the examples. The complement of SEQ ID NO:49, represented herein by SEQ ID NO:51, refers to the nucleic acid sequence of the strand that is fully complementary to the strand having SEQ ID NO:49, which can be easily be determined by those skilled in the art. Likewise, a

nucleic acid sequence complement of any nucleic acid sequence of the present invention refers to the nucleic acid sequence of the nucleic acid strand that is fully complementary to, i.e. can form a complete double helix with, the strand for which the sequence is cited. It should be noted that since nucleic acid sequencing technology is not entirely error-free, SEQ ID NO:49, as well as other nucleic acid and protein sequences presented herein, represents an apparent nucleic acid sequence of the nucleic acid molecule encoding a IL-13Rα1 protein of the present invention.

The nucleic acid sequences of the coding strand and complementary strand of nCaIL-13Rα1$_{1547}$ are represented herein as SEQ ID NO:49 and SEQ ID NO:51, respectively. Translation of SEQ ID NO:49 suggests that nucleic acid molecule nCa IL-13Rα1$_{1547}$ encodes a non full-length PCaIL-13Rα1 protein of about 405 amino acids, denoted herein as PCa IL-13Rα1$_{405}$, the amino acid sequence of which is presented in SEQ ID NO:50, assuming an open reading frame having an initiation codon spanning from nucleotide 1 through nucleotide 3 of SEQ ID NO:49 and a stop codon spanning from nucleotide 1216 through nucleotide 1218 of SEQ ID NO:49. Translation of SEQ ID NO:49 yields SEQ ID NO:50 and a double-stranded nucleic acid molecule representing the region encoding PCa IL-13Rα1$_{405}$ is denoted herein as nCa IL-13Rα1$_{1215}$, represented by SEQ ID NO:52 (coding strand) and SEQ ID NO:53 (complementary strand).

Another embodiment of the present invention is a canine IL-13Rα2 nucleic acid molecule that includes one or more of the following nucleic acid sequences:

(a) the nucleic acid sequence SEQ ID NO:54, SEQ ID NO:57, SEQ ID NO:60, SEQ ID NO:63 and/or SEQ ID NO:65, and/or

(b) the respective complements of these nucleic acid sequences, i.e. SEQ ID NO:56, SEQ ID NO:59, SEQ ID NO:62, SEQ ID NO:64 and/or SEQ ID NO:67, respectively.

These nucleic acid sequences are further described herein. For example, nucleic acid sequence SEQ ID NO:54 and SEQ ID NO:57 encode partial length IL-13Rα2 proteins. SEQ ID NO:60 represents the deduced sequence of the coding strand of a canine cDNA, canine IL-13Rα2 nucleic acid molecule nCaIL-13Rα2$_{1454}$ the cloning of which is disclosed in the examples.

The nucleic acid sequences of the coding strand and complementary strand of nCaIL-13Rα2$_{1454}$ are represented herein as SEQ ID NO:60 and SEQ ID NO:62, respectively. Translation of SEQ ID NO:60 suggests that nucleic acid molecule nCaIL-13Rα2$_{1454}$ encodes a full-length PCaIL-13Rα2 protein of about 386 amino acids, denoted herein as PCa IL-13Rα2$_{386}$, the amino acid sequence of which is presented in SEQ ID NO:61, assuming an open reading frame having an initiation codon spanning from nucleotide 184 through nucleotide 186 of SEQ ID NO:60 and a stop codon spanning from nucleotide 1341 through nucleotide 1343 of SEQ ID NO:60. Translation of SEQ ID NO:60 yields SEQ ID NO:61, and a double-stranded nucleic acid molecule representing the region encoding PCa IL-13Rα2$_{386}$ is denoted herein as nCa IL-13Rα2$_{1158}$, represented by SEQ ID NO:63 (coding strand) and SEQ ID NO:64 (complementary strand). Translation of the putative extracellular domain extending from about residue 22 to about residue 338 of SEQ ID NO:60, represented herein by SEQ ID NO:68 yields SEQ ID NO:69 encoding PCa IL-13Rα2$_{318}$ the protein of the extracellular domain. It is to be noted that SEQ ID NO:68 actually contains an ATG prior to the nucleotides encoding the extracellular domain; as such SEQ IDNO:69 represents the amino acids of the extracellular domain plus an initiation methionine. The natural extra-cellular domain of IL-13Rα2 is actually a 317 amino acid protein with an amino acid sequence spanning 2-318 of SEQ ID NO:69.

Yet another embodiment of the present invention is an IL-13Rα1 nucleic acid molecule that can be an allelic variant that includes a similar but not identical sequence to SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:52 and/or SEQ ID NO:53 and/or any other IL-13Rα1 nucleic acid sequence cited herein.

In another embodiment, an IL-13Raα2 nucleic acid molecule can be an allelic variant that includes a similar but not identical sequence to SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64,SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68 and SEQ ID NO:70 and/or any other IL-13Rα2 nucleic acid sequence cited herein.

In one embodiment, an IL-13Rα1 proteins of the present invention is encoded by a nucleic acid molecule comprising an apparent full-length IL-13Rα1 coding regions, i.e.,

a nucleic acid molecule encoding an apparent full-length IL-13Rα1 protein. Another embodiment of a preferred IL-13Rα1 protein is a fragment thereof encoded by a nucleic acid molecule encoding a protein that includes the low affinity IL-13Rα1 binding site.

In another embodiment, a preferred IL-13Rα protein of the present invention is encoded by a nucleic acid molecule comprising an apparent full-length IL-13Rα2 coding region, i.e., a nucleic acid molecules encoding an apparent full-length IL-13Rα2 protein. Another embodiment of a preferred IL-13Rα2 protein is a fragment thereof encoded by a nucleic acid molecule encoding a protein that includes the high affinity IL-13 binding site.

One embodiment of the present invention is an isolated canine IL-13Rα nucleic acid molecule. Preferred is an isolated canine IL-13Rα1 nucleic acid molecule or an isolated canine IL-13Rα2 nucleic acid molecule. Such a nucleic acid molecule can be RNA, DNA, or a modification thereof. An IL-13Rα nucleic acid of the present invention can be a full-length nucleic acid molecule of a homologue thereof. An IL-13Rα nucleic acid molecule can be single stranded or double stranded. An IL-13Rα nucleic acid molecule of the present invention can e full-length gene, a full-length mRNA or cDNA (complementary DNA) or any portion thereof. A preferred nucleic acid molecule encodes an IL-13Rα protein of the present invention. Such a nucleic acid molecule can encode a full-length protein, mature protein, extracellular domain, or any portion thereof.

It is known in the art that there are commercially available computer programs for determining the degree of similarity between two nucleic acid sequences. These computer programs include various known methods to determine the percentage identity and the number and length of gaps between hybrid nucleic acid molecules. Preferred methods to determine the percent identity among amino acid sequences and also among nucleic acid sequences include analysis using one or more of the commercially available computer programs designed to compare and analyze nucleic acid or amino acid sequences. These computer programs include, but are not limited to, GCG™ (available from Genetics Computer Group, Madison, WI), DNAsis™ (available from Hitachi Software, San Bruno, CA) and MacVector™ (available from the Eastman Kodak Company, New Haven, CT). A preferred method to determine percent identity among

amino acid sequences and also among nucleic acid sequences includes using the Compare function by maximum matching within the program DNAsis Version 2.1 using default parameters. A nucleic acid sequence of the present invention may have at least 85%, preferably at least 90%, and more preferably at least 95%, or even more preferably 100% sequence identity with a nucleic acid molecule in the sequence listing.

Additional preferred canine IgG nucleic acid molecules comprising a nucleic acid sequence that is preferably at least about at least 45% identical, more preferably about at least 50% identical, more preferably about at least 55% identical, more preferably about at least 60% identical, more preferably about at least 65% identical, more preferably about at least 70% identical, more preferably about at least 75% identical, more preferably about at least 80% identical, more preferably about at least 85% identical, more preferably about at least 90% identical and even more preferably about at least 95% identical to a nucleic acid sequence selected from the exemplified sequences (e.g. SEQ ID Nos 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37). Hinge region sequences preferred are those exemplified (e.g. SEQ ID Nos 1, 7, 13, 22). Heavy chain IgG -encoding nucleic acid sequences are are preferred, with the exemplified sequences being most preferred (e.g. SEQ ID Nos 4, 10, 16, 19, 28, 31, 34 and 37). Light chain canine IgG sequences are also provided, particularly those exemplified (e.g. SEQ ID NO 25). Particularly preferred are nucleic acid molecules comprising the exemplified sequences. Also preferred are fragments of any of such nucleic acid molecules. Percent identity may be determined using the Compare function by maximum matching within the program DNAsis Version 2.1 using default parameters.

Stringent hybridization conditions are determined based on defined physical properties of the nucleic acid molecule to which the nucleic acid molecule to be compared is being hybridized, and can be defined mathematically. Stringent hybridization conditions are those experimental parameters that allow an individual skilled in the art to identify significant similarities between heterologous nucleic acid molecules. These conditions are well known to those skilled in the art. See, for example, Sambrook, *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, and Meinkoth, *et al.*, 1984, *Anal. Biochem. 138,* 267-284, each of which is incorporated by reference herein in its entirety. As explained in detail in the cited

references, the determination of hybridization conditions involves the manipulation of a set of variables including the ionic strength (M, in moles/liter), the hybridization temperature (°C), the concentration of nucleic acid helix destabilizing agents (such as formamide), the average length of the shortest hybrid duplex (n), and the percent G+C composition of the fragment to which an unknown nucleic acid molecule is being hybridized. For nucleic acid molecules of at least about at least 150 nucleotides, these variables are inserted into a standard mathematical formula to calculate the melting temperature, or T_m, of a given nucleic acid molecule. As defined in the formula below, T_m is the temperature at which two complementary nucleic acid molecule strands will disassociate, assuming 100% complementarity between the two strands: T_m=81.5°C+16.6 log M+0.41(%G+C)-500/n-0.61(% formamide). For nucleic acid molecules smaller than about 50 nucleotides, hybrid stability is defined by the dissociation temperature (T_d), which is defined as the temperature at which 50% of the duplexes dissociate. For these smaller molecules, the stability at a standard ionic strength is defined by the following equation: T_d=4(G+C)+2(A+T). A temperature of 5°C below T_d is used to detect hybridization between perfectly matched molecules.

Hybridization reactions are often carried out by attaching the nucleic acid molecule to be hybridized to a solid support such as a membrane, and then hybridizing with a labeled nucleic acid molecule, typically referred to as a probe, suspended in a hybridization solution. Examples of common hybridization reaction techniques include, but are not limited to, the well-known Southern and northern blotting procedures. Typically, the actual hybridization reaction is done under non-stringent conditions, e.g., at a lower temperature and/or a higher salt concentration, and then high stringency is achieved by washing the membrane in a solution with a higher temperature and/or lower salt concentration in order to achieve the desired stringency.

For example, if the skilled artisan wished to identify a nucleic acid molecule that hybridized under stringent hybridization conditions with a canine nucleic acid molecule of about 150 bp in length, the following conditions could preferably be used. The average G+C content of canine genome is about 53%. The unknown nucleic acid molecules would be attached to a support membrane, and the 150 bp probe would be labeled, e.g. with a radioactive tag. The hybridization reaction could be carried out in a

solution comprising 2X SSC and 0% formamide, at a temperature of about 37°C (low stringency conditions). Solutions of differing concentrations of SSC can be made by one of skill in the art by diluting a stock solution of 20X SSC (175.3 gram NaCl and about 88.2 gram sodium citrate in 1 liter of water, pH 7) to obtain the desired concentration of SSC. In order to achieve high stringency hybridization, the skilled artisan would calculate the washing conditions required to allow up to 30% base-pair mismatch. For example, in a wash solution comprising 1X SSC and 0% formamide, the T_m of perfect hybrids would be about 86.3°C:

$$81.5°C+16.6 \log (.15M) + (.41 \times 53) - (500/150) - (0.61 \times 0) = 86.3°C.$$

The present invention also includes nucleic acid molecules that are oligonucleotides capable of hybridizing, under stringent hybridization conditions, with complementary regions of other, preferably longer, nucleic acid molecules of the present invention such as those comprising canine IgG (heavy and/or light chain) genes or other canine IgG (heavy and/or light chain) nucleic acid molecules. Oligonucleotides of the present invention can be RNA, DNA, or derivatives of either. The minimum size of such oligonucleotides is the size required for formation of a stable hybrid between an oligonucleotide and a complementary sequence on a nucleic acid molecule of the present invention. Minimal size characteristics are disclosed herein. The present invention includes oligonucleotides that can be used as, for example, probes to identify nucleic acid molecules, primers to produce nucleic acid molecules. The present invention also includes the use of such oligonucleotides to protect animals from disease using one or more of such technologies. Appropriate oligonucleotide-containing therapeutic compositions can be administered to an animal using techniques known to those skilled in the art.

Also well known to those skilled in the art is how base-pair mismatch, e.g. differences between two nucleic acid molecules being compared, including non-complementarity of bases at a given location, and gaps due to insertion or deletion of one or more bases at a given location on either of the nucleic acid molecules being compared, will affect T_m or T_d for nucleic acid molecules of different sizes. For example, T_m

decreases about 1°C for each 1% of mismatched base-pairs for hybrids greater than about 150 bp, and T_d decreases about 5°C for each mismatched base-pair for hybrids below about 50 bp. Conditions for hybrids between about 50 and about 150 base-pairs can be determined empirically and without undue experimentation using standard laboratory procedures well known to those skilled in the art. These simple procedures allow one skilled in the art to set the hybridization conditions (by altering, for example, the salt concentration, the formamide concentration or the temperature) so that only nucleic acid hybrids with less than a specified % base-pair mismatch will hybridize. Stringent hybridization conditions are commonly understood by those skilled in the art to be those experimental conditions that will allow hybridization between molecules having about 30% or less base-pair mismatch (e.g., about 70% or greater identity). Because one skilled in the art can easily determine whether a given nucleic acid molecule to be tested is less than or greater than about 50 nucleotides, and can therefore choose the appropriate formula for determining hybridization conditions, he or she can determine whether the nucleic acid molecule will hybridize with a given gene under stringent hybridization conditions and similarly whether the nucleic acid molecule will hybridized under conditions designed to allow a desired amount of base pair mismatch.

Thus, based on the equation given previously, to achieve hybridization with nucleic acid molecules having about 30% base-pair mismatch, hybridization washes would be carried out at a temperature of about 56.3°C. It is thus within the skill of one in the art to calculate additional hybridization temperatures based on the desired percentage base-pair mismatch, formulae and G/C content disclosed herein. For example, it is appreciated by one skilled in the art that as the nucleic acid molecule to be tested for hybridization against nucleic acid molecules of the present invention having sequences specified herein becomes longer than 150 nucleotides, the T_m for a hybridization reaction allowing up to 30% base-pair mismatch will not vary significantly from 56.3°C.

In one embodiment of the present invention, a preferred canine IgG (heavy and/or light chain) nucleic acid molecule includes an isolated nucleic acid molecule which hybridizes under conditions which preferably allow about 50% or less base pair mismatch, more preferably under conditions which allow about 45% or less base pair mismatch, more preferably under conditions which allow about 40% or less base pair

mismatch, more preferably under conditions which allow about 35% or less base pair mismatch, more preferably under conditions which allow about 30% or less base pair mismatch, more preferably under conditions which allow about 25% or less base pair mismatch, more preferably under conditions which allow about 20% or less base pair mismatch, more preferably under conditions which allow about 15% or less base pair mismatch, more preferably under conditions which allow about 10% or less base pair mismatch and even more preferably under conditions which allow about 5% or less base pair mismatch with a nucleic acid molecule selected from the exemplified compounds.

The present invention also provides isolated nucleic acid molecules, wherein said nucleic acid molecules comprise a nucleic acid sequence selected from the group consisting of:

(a) a nucleic acid sequence comprising at least 70 contiguous nucleotides of the sequence shown in the sequence selected from the group consisting of SEQ ID NO 1, SEQ ID NO 7 and SEQ ID NO 13.

(b) a nucleic acid sequence comprising at least 350 contiguous nucleotides of the sequence shown in the sequence selected from the group consisting of SEQ ID NO 4, SEQ ID NO 10, and SEQ ID NO 16

(c) a nucleic acid sequence comprising at least 450 contiguous nucleotides of the sequence shown in SEQ ID NO 19,

(d) a nucleic acid sequence which encodes an amino acid comprising at least 20 contiguous residues of the sequence shown in SEQ ID NO 2, and SEQ ID NO 14,

(d) a nucleic acid sequence which encodes an amino acid comprising at least 100 contiguous residues of the sequence selected from the sequences shown in SEQ ID NO 5, SEQ ID NO 8, SEQ ID NO 11, and SEQ ID NO 17,

(e) a nucleic acid sequence which encodes an amino acid comprising at least 200 contiguous residues of the sequence shown in SEQ ID NO 20,

(f) a nucleic acid sequence fully complementary to a nucleic acid sequence selected from the group consisting of a nucleic acid sequence of (a), a nucleic acid sequence of (b), a nucleic acid sequence of (c), a nucleic acid

sequence of (d), a nucleic acid sequence of (e), and a nucleic acid sequence of (f).

Also provided are isolated nucleic acid molecules, wherein said nucleic acid molecules comprise a nucleic acid sequence selected from the group consisting of

(a) a nucleic acid sequence comprising at least 150 contiguous nucleotides of the sequence shown in the sequence selected from the group consisting of SEQ ID NO 1, and SEQ ID NO 13.

(b) a nucleic acid sequence comprising at least 500 contiguous nucleotides of the sequence shown in the sequence selected from the group consisting of SEQ ID NO 4, SEQ ID NO 7, SEQ ID NO 10, and SEQ ID NO 16

(c) a nucleic acid sequence comprising at least 700 contiguous nucleotides of the sequence shown in SEQ ID NO 19,

(d) a nucleic acid sequence which encodes an amino acid comprising at least 50 contiguous residues of the sequence shown in SEQ ID NO 2, and SEQ ID NO 14,

(e) a nucleic acid sequence which encodes an amino acid comprising at least 200 contiguous residues of the sequence selected from the sequences shown in SEQ ID NO 5, SEQ ID NO 8, SEQ ID NO 11, and SEQ ID NO 17,

(f) a nucleic acid sequence which encodes an amino acid comprising at least 300 contiguous residues of the sequence shown in SEQ ID NO 20,

(g) a nucleic acid sequence fully complementary to a nucleic acid sequence selected from the group consisting of a nucleic acid sequence of (a), a nucleic acid sequence of (b), a nucleic acid sequence of (c), a nucleic acid sequence of (d), a nucleic acid sequence of (e), a nucleic acid sequence of (f), and a nucleic acid sequence of (g).

Also provided are isolated nucleic acid molecules, wherein said nucleic acid molecules comprise a nucleic acid sequence selected from the group consisting of

(a) a nucleic acid sequence which is selected from the group consisting of

SEQ ID NO 1, SEQ ID NO 4, SEQ ID NO 7, SEQ ID NO 10, SEQ ID NO 13, SEQ ID NO 16, and SEQ ID NO 19,

(b) a nucleic acid sequence which is selected from the group consisting of SEQ ID NO 2, SEQ ID NO 5, SEQ ID NO 8, SEQ ID NO 111, SEQ ID NO 14, SEQ ID NO 17, and SEQ ID NO 20,

(c) a nucleic acid sequence fully complementary to a nucleic acid sequence selected from the group consisting of a nucleic acid sequence of (a), and a nucleic acid sequence of (b).

Another embodiment of the present invention is a preferred canine IL-13Rα1 nucleic acid molecule that includes a isolated nucleic acid molecule of at least 75 nucleotides in length or (b) an isolated nucleic acid molecule that hybridizes under conditions which allow less than or equal to about 10% base pair mismatch, and even more preferably under conditions which allow less than or equal to about 5% base pair mismatch with a nucleic acid molecule selected from the group consisting of SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:52 and/or SEQ ID NO:53, and/or a fragment thereof having at least 80 nucleotides.

Preferred canine IL-13Rα1 nucleic acid molecules of the present invention include nucleic acid molecules comprising a nucleic acid sequence that is preferably at least about 90%, more preferably at least about 92%, more preferably about 94%, more preferably about 96%, and even more preferably at least about 98% identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:52 and/or SEQ ID NO:53. Also preferred are fragments of any of such nucleic acid molecules, particularly those that are at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 100 nucleotides, at least about 120 nucleotides, at least about 140 nucleotides, at least about 160 nucleotides, at least about 180 nucleotides, at least about 200 nucleotides, at least about 250 nucleotides, at least about 300 nucleotides, at least about 350 nucleotides, or at least about 400 nucleotides at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, at least about 1000 nucleotides, at least

about 1100 nucleotides, at least about 1300 nucleotides, or at least about 1500 nucleotides. Percent identity is determined by the DNAsis™ computer program with the gap penalty set at 5, the number of top diagonals set at 5, the fixed gap penalty set at 10, the k-tuple set at 2, the window size set at 10 and the floating gap penalty set at 10.

One embodiment of the present invention is a canine IL-13Rα1 nucleic acid molecule that includes an isolated nucleic acid molecule of at least 75 nucleotides that hybridizes under conditions which allow less than or equal to about 10% base pair mismatch, and even more preferably under conditions which allow less than or equal to about 5% base pair mismatch with a nucleic acid molecule selected from the group consisting of SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:52 and/or SEQ ID NO:53, and/or a fragment thereof having at least 80 nucleotides. Also preferred are fragments of at least 85, at least 90, at least 95, at least 100, at least 110, at least 120, at least 140, at least 160, at least 180, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, at least 500, at least 550, at least 600, at least 650, at least 700, at least 750, at least 800, at least 850, at least 900, at least 950, at least 1000, at least 1100, at least 1200, at least 1300, at least 1400, at least 1500, or at least 1600 nucleotides in length.

Preferred canine IL-13Rα1 nucleic acid molecules of the present invention include nucleic acid molecules comprising a nucleic acid sequence that is preferably at least about 90%, more preferably at least about 92%, more preferably about 94%, more preferably about 96%, and even more preferably at least about 98% identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:52 and/or SEQ ID NO:53. Also preferred are oligonucleotides of any of such nucleic acid molecules, particularly those that are at least about 80 nucleotides. Percent identity is determined by the DNAsis™ computer program with the gap penalty set at 5, the number of top diagonals set at 5, the fixed gap penalty set at 10, the k-tuple set at 2, the window size set at 10 and the floating gap penalty set at 10.

Another embodiment of a canine IL-13Rα1 nucleic acid molecule of the present invention is

(a) an isolated nucleic acid molecule comprising a nucleic acid sequence

encoding a protein selected from the group consisting of: (i) a protein that is at least 85, at least 90, at least 95, or at least 100 percent identical in sequence to amino acid sequence SEQ ID NO:50, wherein said percent identity is determined by the DNAsis™ computer program with the gap penalty set at 5, the number of top diagonals set at 5, the fixed gap penalty set at 10, the k-tuple set at 2, the window size set at 10 and the floating gap penalty set at 10; and (ii) a protein comprising a fragment of at least 45 contiguous amino acids identical in sequence to an at least 45 contiguous amino acid sequence of a protein of (a);

(b) an isolated nucleic acid molecule comprising a nucleic acid sequence encoding a protein that comprises an at least 40 contiguous amino acid region identical in sequence to an at least 40 contiguous amino acid region of SEQ ID NO:50; and

(c) an isolated nucleic acid molecule fully complementary to any of the nucleic acid molecules of (a) or (b). Also preferred are fragments that are at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 100, at least 120, at least 140, at least 160, at least 180, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, at least 500, or at least 550 amino acids in length.

One embodiment of the present invention is an IL-13Rα2 nucleic acid molecule that includes at least one of the following: (a) an isolated nucleic acid molecule including at least 40 contiguous nucleotides identical in sequence to an at least 40 contiguous nucleotide region of at least one of the following nucleic acid sequences: SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64,SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68 and SEQ ID NO:70; and (b) an isolated nucleic acid molecule including a nucleic acid sequence that is at least 80, at least 85, at least 90, at least 95, at least 100 percent identical in sequence to at least one of the following nucleic acid sequences: SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64,SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68 and SEQ ID NO:70, and a fragment thereof, wherein the fragment is at least 50

nucleotides in length, and wherein percent identity is determined by the DNAsis™ computer program with the gap penalty set at 5, the number of top diagonals set at 5, the fixed gap penalty set at 10, the k-tuple set at 2, the window size set at 10 and the floating gap penalty set at 10. In one embodiment, each of these nucleic acid molecule is a canine IL-13Rα2 nucleic acid molecule. In another embodiment, such nucleic acid molecules do not hybridize under conditions comprising hybridization at 65°C in 0.1 X SSC followed by washing at 65°C in 0.1 X SSC with a nucleic acid sequence selected from the group consisting of SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97 and SEQ ID NO:98. In one embodiment, such nucleic acid molecules do not hybridize under conditions comprising hybridization at 52°C in 5 X SSC followed by washing at 52°C in 2 X SSC with a nucleic acid sequence selected from the group consisting of SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97 and SEQ ID NO:98, unless detection of hybridization requires a long time to detect, for example, because the signal is so low as to resemble background. Also preferred are fragments that are at least 45, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 100, at least 120, at least 140, at least 160, at least 180, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, at least 500, at least 550, at least 600, at least 650, at least 700, at least 750, at least 800, at least 850, at least 900, at least 950, at least 1000, at least 1100, at least 1200, at least 1300, at least 1400, at least 1500, or at least 1600 nucleotides in length.

One embodiment of the present invention is a canine IL-13Rα2 nucleic acid molecule that hybridizes under conditions which allow less than or equal to about 20% base pair mismatch, preferably under conditions which allow less than or equal to about 15% base pair mismatch, more preferably under conditions which allow less than or equal to about 10% base pair mismatch, more preferably under conditions which allow less than or equal to about 5% base pair mismatch with a nucleic acid molecule selected from the group consisting of SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64,SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68 and SEQ ID NO:70.

Another embodiment of the present invention is an isolated nucleic acid molecule selected from the group consisting of: (a) an isolated nucleic acid molecule comprising a

nucleic acid sequence encoding a protein selected from the group consisting of:(i) a protein comprising an amino acid sequence that is at least 70, at least 75, at least 80, at least 85, at least 90, at least 95 or at least 100 percent identical in sequence to an amino acid sequence selected from the group consisting of SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:61, SEQ ID NO:66, and SEQ ID NO:69, wherein percent identity is determined by the DNAsis™ computer program with the gap penalty set at 5, the number of top diagonals set at 5, the fixed gap penalty set at 10, the k-tuple set at 2, the window size set at 10 and the floating gap penalty set at 10, and ii) a protein comprising a fragment of at least 40 contiguous amino acids identical in sequence to an at least 40 contiguous amino acid sequence of a protein of (a)(i); (b) an isolated nucleic acid molecule comprising a nucleic acid sequence encoding a protein that comprises an at least 30 contiguous amino acid region identical in sequence to an at least 30 contiguous amino acid region of SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:61, SEQ ID NO:66, and SEQ ID NO:69; and (c) an isolated nucleic acid molecule fully complementary to any of the nucleic acid molecules of (a) or (b). In one embodiment, each of these nucleic acid molecule is a canine IL-13Rα2 nucleic acid molecule. In another embodiment, such nucleic acid molecules do not hybridize under conditions comprising hybridization at 65°C in 0.1 X SSC followed by washing at 65°C in 0.1 X SSC with a nucleic acid sequence selected from the group consisting of SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97 and SEQ ID NO:98. In one embodiment, such nucleic acid molecules do not hybridize under conditions comprising hybridization at 52°C in 5 X SSC followed by washing at 52°C in 2 X SSC with a nucleic acid sequence selected from the group consisting of SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97 and SEQ ID NO:98, unless detection of hybridization requires a long time to detect, for example, because the signal is so low as to resemble background. Also preferred are fragments that are at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 100, at least 120, at least 140, at least 160, at least 180, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, at least 500, or at least 550 amino acids in length.

Another embodiment of the present invention, as discussed elsewhere herein, is a chimeric nucleic acid molecule that includes a nucleic acid molecule encoding a carrier

protein domain and a nucleic acid molecule encoding a CaIL-13Rα protein domain. A nucleic acid molecule encoding a CaIL-13Rα protein domain can be any CaIL-13Rα protein-encoding nucleic acid molecule of the present invention, including any CaIL-13Rα1 protein-encoding nucleic acid molecule of the present invention or any CaIL-13Rα2 protein-encoding nucleic acid molecule of the present invention. The present invention also includes nucleic acid molecules fully complementary to the coding strands of such chimeric nucleic acid molecules.

One embodiment of the present invention is a nucleic acid molecule comprising all or part of nucleic acid molecules nCaIL-13Rα1$_{483}$, nCaIL-13Rα1$_{1547}$, nCaIL-13Rα1$_{1215}$, nCaIL-13Rα2$_{620}$, nCaIL-13Rα2$_{878}$, nCaIL-13Rα2$_{1454}$, nCaIL-13Rα2$_{1158}$, nCaIL-13Rα2$_{1098}$, nCaIL-13Rα2$_{954}$, nCaIL-13Rα2-Fc-3523, nCaIL-13Rα2-Fc-4325, nCaIL-13Rα2-Fc-B9, nCaIL-13Rα2-Fc-B8, or allelic variants of any of these nucleic acid molecules. As such, a preferred nucleic acid molecule of the present invention includes at least a portion of nucleic acid sequence SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:57, SEQ ID NO:60, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:68, SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:77 and/or SEQ ID NO:80 as well as allelic variant of any of these listed nucleic acid molecules. Also included in the present invention are other homologs of nucleic acid molecules having these nucleic acid sequences; preferably such a homolog encodes or is complementary to a nucleic acid molecule that encodes at least one epitope that elicits an immune response against a protein having amino acid sequence SEQ ID NO:50, SEQ ID NO:55, SEQ ID NO:58,SEQ ID NO:61, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:72, SEQ ID NO:75, SEQ ID NO:78 and/or SEQ ID NO:81. Such nucleic acid molecules can include nucleotides in addition to those included in the SEQ ID NOs, such as, but not limited to, a full-length gene, a full-length coding region, a nucleic acid molecule encoding a fusion protein, or a nucleic acid molecule encoding a multivalent protective compound, such as a multivalent vaccine. The present invention also includes nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

In another embodiment, a preferred canine IL-13Rα1 nucleic acid molecule encodes a IL-13Rα1 protein at least about 45 amino acids, at least about 100 amino acids,

at least about 120 amino acids, at least about 150 amino acids, at least about 170 amino acids, at least about 190 amino acids, at least about 210 amino acids in length, at least about 250 amino acids in length, at least about 350 amino acids in length, or about 405 amino acids in length.

In another embodiment, a preferred canine IL-13Rα2 nucleic acid molecule encodes a IL-13Rα2 protein at least about 40 amino acids, at least about 100 amino acids, at least about 120 amino acids, at least about 150 amino acids, at least about 170 amino acids, at least about 190 amino acids, at least about 210 amino acids in length, at least about 250 amino acids in length, at least about 350 amino acids in length, or about 386 amino acids in length.

A preferred canine nucleic acid molecule comprises a nucleic acid sequence that encodes at least a portion of a canine IL-13Rα2 protein that is capable of binding to IL-13Rα2 ligand(s) as disclosed herein. Preferably, such a nucleic acid molecule encodes a protein having SEQ ID NO:50, SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:61, SEQ ID NO:66, and/or SEQ ID NO:69, or allelic variants thereof.

The present invention also includes nucleic acid molecules that are oligonucleotides capable of hybridizing, under stringent hybridization conditions, with complementary regions of other, preferably longer, canine IL-13Rα nucleic acid molecules of the present invention. Oligonucleotides of the present invention can be RNA, DNA, or derivatives of either. The minimum size of such oligonucleotides is the size required for formation of a stable hybrid between an oligonucleotide and a complementary sequence on a nucleic acid molecule of the present invention, typically from about 12 to 15 to about 17 to 18 nucleotides depending on the GC/AT content. A preferred oligonucleotide of the present invention has a maximum size of from about 100 to about 200 nucleotides. The present invention includes oligonucleotides that can be used as, for example, probes to identify nucleic acid molecules, primers to produce nucleic acid molecules, or therapeutic reagents to inhibit canine IL-13Rα protein production or activity, e.g., as antisense-, triplex formation-, ribozyme- and/or RNA drug-based reagents. The present invention also includes the use of such oligonucleotides to protect animals from disease using one or more of such technologies. Appropriate oligonucleotide-containing therapeutic compositions can be administered to

an animal using techniques known to those skilled in the art.

Knowing the nucleic acid sequences of certain canine IgG (heavy and/or light chain) nucleic acid molecules and canine IL-13Rα nucleic acid molecules of the present invention allows one skilled in the art to, for example, (a) make copies of those nucleic acid molecules, (b) obtain nucleic acid molecules including at least a portion of such nucleic acid molecules (e.g., nucleic acid molecules including full-length genes, full-length coding regions, regulatory control sequences, truncated coding regions), (c) obtain canine IgG (heavy and/or light chain) nucleic acid molecules and/or canine IL-13Rα nucleic acid molecules from other species, and (d) construct fusion nucleic acid molecules comprising canine IgG (heavy and/or light chain) and canine IL-13Rα nucleic acid sequences. Such nucleic acid molecules can be obtained in a variety of ways including screening appropriate expression libraries with antibodies of the present invention; traditional cloning techniques using oligonucleotide probes of the present invention to screen appropriate libraries of DNA; and PCR amplification of appropriate libraries or DNA using oligonucleotide primers of the present invention. Preferred libraries to screen or from which to amplify nucleic acid molecules include canine cDNA libraries as well as genomic DNA libraries. Similarly, preferred DNA sources to screen or from which to amplify nucleic acid molecules include canine adult cDNA and genomic DNA. Techniques to clone and amplify genes are disclosed, for example, in Sambrook et al., *ibid.*

One embodiment of the present invention includes a recombinant vector, which includes at least one isolated nucleic acid molecule of the present invention, inserted into any vector capable of delivering the nucleic acid molecule into a host cell. Such a vector contains heterologous nucleic acid sequences, that is nucleic acid sequences that are not naturally found adjacent to nucleic acid molecules of the present invention and that preferably are derived from a species other than the species from which the nucleic acid molecule(s) are derived. The vector can be either RNA or DNA, either prokaryotic or eukaryotic, and typically is a virus or a plasmid. Recombinant vectors can be used in the cloning, sequencing, and/or otherwise manipulation of canine IgG (heavy and/or light chain) nucleic acid molecules and /or canine IL-13Rα nucleic acid molecules of the present invention.

One type of recombinant vector, referred to herein as a recombinant molecule, comprises a nucleic acid molecule of the present invention operatively linked to an expression vector. The phrase "operatively linked" refers to insertion of a nucleic acid molecule into an expression vector in a manner such that the molecule is able to be expressed when transformed into a host cell. As used herein, an expression vector is a DNA or RNA vector that is capable of transforming a host cell and of effecting expression of a specified nucleic acid molecule. Preferably, the expression vector is also capable of replicating within the host cell. Expression vectors can be either prokaryotic or eukaryotic, and are typically viruses or plasmids. Expression vectors of the present invention include any vectors that function (e.g., direct gene expression) in recombinant cells of the present invention, including in bacterial, fungal, endoparasite, insect, other animal, and plant cells. Preferred expression vectors of the present invention can direct gene expression in bacterial, yeast, insect and mammalian cells and more preferably in the cell types disclosed herein.

In particular, recombinant molecules of the present invention contain regulatory sequences such as transcription control sequences, translation control sequences, origins of replication, and other regulatory sequences that are compatible with the recombinant cell and that control the expression of nucleic acid molecules of the present invention. In particular, recombinant molecules of the present invention include transcription control sequences. Transcription control sequences are sequences which control the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which control transcription initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences include any transcription control sequence that can function in at least one of the recombinant cells of the present invention. A variety of such transcription control sequences are known to those skilled in the art. Preferred transcription control sequences include those which function in bacterial, yeast, insect and mammalian cells, such as, but not limited to, *tac*, *lac*, *trp*, *trc*, oxy-pro, omp/lpp, rrnB, bacteriophage lambda (such as lambda p_L and lambda p_R and fusions that include such promoters), bacteriophage T7, T7*lac*, bacteriophage T3, bacteriophage SP6, bacteriophage SP01, metallothionein, alpha-mating factor, *Pichia* alcohol oxidase, alphavirus subgenomic promoters (such as Sindbis virus

subgenomic promoters), antibiotic resistance gene, baculovirus, *Heliothis zea* insect virus, vaccinia virus, herpesvirus, raccoon poxvirus, other poxvirus, adenovirus, cytomegalovirus (such as intermediate early promoters), simian virus 40, retrovirus, actin, retroviral long terminal repeat, Rous sarcoma virus, heat shock, phosphate and nitrate transcription control sequences as well as other sequences capable of controlling gene expression in prokaryotic or eukaryotic cells. Additional suitable transcription control sequences include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (e.g., promoters inducible by interferons or interleukins). Transcription control sequences of the present invention can also include naturally occurring transcription control sequences naturally associated with dogs.

Suitable and preferred nucleic acid molecules to include in recombinant vectors of the present invention are as disclosed herein. Preferred nucleic acid molecules to include in recombinant vectors include canine IgG, and fragments thereof, canine IL-13Rα as well as fusions of canine IgG and canineIL-13Rα nucleic acid sequences. Particularly preferred nucleic acid molecules to include in recombinant vectors, and particularly in recombinant molecules, include nCaIL-13Rα1_{483}, nCaIL-13Rα1_{1547}, nCaIL-13Rα1_{1215}, nCaIL-13Rα2_{620}, nCaIL-13Rα2_{878}, nCaIL-13Rα2_{1454}, nCaIL-13Rα2_{1158}, nCaIL-13Rα2_{1098}, nCaIL-13Rα2_{954}, nCaIL-13Rα2-Fc-3523, nCaIL-13Rα2-Fc-4325, nCaIL-13Rα2-Fc-B9,and nCaIL-13Rα2-Fc-B8.

Recombinant molecules of the present invention may also (a) contain secretory signals (e.g., signal segment nucleic acid sequences) to enable an expressed canine IgG (heavy and/or light chain) protein and /or canine IL-13Rα proteins of the present invention to be secreted from the cell that produces the protein and/or (b) contain fusion sequences which lead to the expression of nucleic acid molecules of the present invention as fusion proteins. Examples of suitable signal segments include any signal segment capable of directing the secretion of a protein of the present invention. Preferred signal segments include, but are not limited to, tissue plasminogen activator (t-PA), interferon, interleukin, growth hormone, histocompatibility and viral envelope glycoprotein signal segments, as well as natural signal segments. Suitable fusion segments encoded by fusion segment nucleic acids are disclosed herein. In addition, a nucleic acid molecule of the present invention can be joined to a fusion segment that directs the encoded protein to

the proteosome, such as a ubiquitin fusion segment. Recombinant molecules may also include intervening and/or untranslated sequences surrounding and/or within the nucleic acid sequences of nucleic acid molecules of the present invention.

Another embodiment of the present invention includes a recombinant cell comprising a host cell transformed with one or more nucleic acid molecules or recombinant molecules of the present invention. Transformation of a nucleic acid molecule into a cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. A recombinant cell may remain unicellular or may grow into a tissue, organ or a multicellular organism. It is to be noted that a cell line refers to any recombinant cell of the present invention that is not a transgenic animal. Transformed nucleic acid molecules of the present invention can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transformed, i.e., recombinant, cell in such a manner that their ability to be expressed is retained.

Preferred nucleic acid molecules with which to transform a cell include canine IgG (heavy and/or light chain) nucleic acid molecules and/or canine IL-13Rα nucleic acid molecules and/or fusions of said nucleic acid molecules disclosed herein. Particularly preferred nucleic acid molecules with which to transform a cell include nCaIL-13Rα1$_{483}$, nCaIL-13Rα1$_{1547}$, nCaIL-13Rα1$_{1215}$, nCaIL-13Rα2$_{620}$, nCaIL-13Rα2$_{878}$, nCaIL-13Rα2$_{1454}$, nCaIL-13Rα2$_{1158}$, nCaIL-13Rα2$_{1098}$, nCaIL-13Rα2$_{954}$, nCaIL-13Rα2-Fc-3523, nCaIL-13Rα2-Fc-4325, nCaIL-13Rα2-Fc-B9, and nCaIL-13Rα2-Fc-B8.

Recombinant DNA technologies can be used to improve expression of transformed nucleic acid molecules by manipulating, for example, the number of copies of the nucleic acid molecules within a host cell, the efficiency with which those nucleic acid molecules are transcribed, the efficiency with which the resultant transcripts are translated, and the efficiency of post-translational modifications. Recombinant techniques useful for increasing the expression of nucleic acid molecules of the present invention include, but are not limited to, operatively linking nucleic acid molecules to high-copy number plasmids, integration of the nucleic acid molecules into one or more

host cell chromosomes, addition of vector stability sequences to plasmids, substitutions or modifications of transcription control signals (e.g., promoters, operators, enhancers), substitutions or modifications of translational control signals (e.g., ribosome binding sites, Shine-Dalgarno sequences), modification of nucleic acid molecules of the present invention to correspond to the codon usage of the host cell, deletion of sequences that destabilize transcripts, and use of control signals that temporally separate recombinant cell growth from recombinant enzyme production during fermentation. The activity of an expressed recombinant protein of the present invention may be improved by fragmenting, modifying, or derivatizing nucleic acid molecules encoding such a protein.

Also provided by the present invention are recombinant cells transformed with a nucleic acid described herein. Suitable host cells to transform include any cell that can be transformed with a nucleic acid molecule of the present invention. Host cells can be either untransformed cells or cells that are already transformed with at least one nucleic acid molecule (e.g., nucleic acid molecules encoding one or more proteins of the present invention and/or other proteins useful in the production of multivalent vaccines). Host cells of the present invention either can be endogenously (e.g., naturally) capable of producing canine IgG (heavy and/or light chain) and/or canine IL-13Ra proteins of the present invention or can be capable of producing such proteins after being transformed with at least one nucleic acid molecule of the present invention. Host cells of the present invention can be any cell capable of producing at least one protein of the present invention, and include bacterial, fungal (including yeast), other insect, other animal and plant cells. Preferred host cells include bacterial, mycobacterial, yeast, parasite, insect and mammalian cells. More preferred host cells include *Salmonella*, *Escherichia*, *Bacillus*, *Listeria*, *Saccharomyces*, *Spodoptera*, *Mycobacteria*, *Trichoplusia*, BHK (baby hamster kidney) cells, MDCK cells (normal dog kidney cell line for canine herpesvirus cultivation), CRFK cells (normal cat kidney cell line for canine herpesvirus cultivation), CV-1 cells (African monkey kidney cell line used, for example, to culture raccoon poxvirus), COS (e.g., COS-7) cells, and Vero cells. Particularly preferred host cells are *Escherichia coli*, including *E. coli* K-12 derivatives; *Salmonella typhi*; *Salmonella typhimurium*, including attenuated strains such as UK-1 χ3987 and SR-11 χ4072; *Spodoptera frugiperda*; *Trichoplusia ni*; BHK cells; MDCK cells; CRFK cells; CV-1

cells; COS cells; Vero cells; and non-tumorIgGnic mouse myoblast G8 cells (e.g., ATCC CRL 1246). Additional appropriate mammalian cell hosts include other kidney cell lines, other fibroblast cell lines (e.g., human, murine or chicken embryo fibroblast cell lines), myeloma cell lines, Chinese hamster ovary cells, mouse NIH/3T3 cells, LMTK^{31} cells and/or HeLa cells. In one embodiment, the proteins may be expressed in myeloma cell lines employing immunoglobulin promoters.

A recombinant cell is preferably produced by transforming a host cell with one or more recombinant molecules, each comprising one or more nucleic acid molecules of the present invention operatively linked to an expression vector containing one or more transcription control sequences. A recombinant molecule of the present invention is a molecule that can include at least one of any nucleic acid molecule heretofore described operatively linked to at least one of any transcription control sequence capable of effectively regulating expression of the nucleic acid molecule(s) in the cell to be transformed, examples of which are disclosed herein.

Recombinant cells of the present invention can also be co-transformed with one or more recombinant molecules including canine IgG and/or canine IL-13Rα nucleic acid molecules encoding one or more proteins of the present invention and one or more other nucleic acid molecules encoding other compounds. Suitable and preferred nucleic acid molecules as well as suitable and preferred recombinant molecules with which to transform cells are disclosed herein.

Another embodiment of the present invention are isolated canine IgG proteins, wherein said proteins comprise an amino acid sequence selected from the group consisting of"

(a) an amino acid sequence encoded by a nucleic acid sequence which has at least 70% identity to a nucleic acid sequence which is selected from the group consisting of SEQ ID NO 1, SEQ ID NO 7, and SEQ ID NO 13, wherein said identity is determined using the DNAsis computer program and default parameters,

(b) an amino acid sequence which has at least 70% identity to an amino acid sequence selected from the group consisting of SEQ ID NO 2, and SEQ ID NO 14, wherein said identity is determined using the DNAsis computer

program and default parameters, and

(c) an amino acid sequence encoded by a nucleic acid sequence which is an allelic variant of a nucleic acid sequence selected from the group consisting of SEQ ID NO 1, SEQ ID NO 7, and SEQ ID NO 13.

Also provided are canine IgG proteins, wherein said proteins comprise an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence encoded by a nucleic acid sequence comprising at least 70 contiguous nucleotides of the sequence shown in the sequence selected from the group consisting of SEQ ID NO 1, and SEQ ID NO 13.

(b) an amino acid sequence encoded by a nucleic acid sequence comprising at least 350 contiguous nucleotides of the sequence shown in the sequence selected from the group consisting of SEQ ID NO 4, SEQ ID NO 7, SEQ ID NO 10, and SEQ ID NO 16

(c) an amino acid sequence encoded by a nucleic acid sequence comprising at least 450 contiguous nucleotides of the sequence shown in SEQ ID NO 19,

(d) an amino acid sequence encoded by a nucleic acid sequence which is selected from the group consisting of SEQ ID NO 1, SEQ ID NO 4, SEQ ID NO 7, SEQ ID NO 10, SEQ ID NO 13, SEQ ID NO 16, and SEQ ID NO 19,

(e) an amino acid comprising at least 20 contiguous residues of the sequence shown in SEQ ID NO 2, and SEQ ID NO 14,

(f) an amino acid comprising at least 100 contiguous residues of the sequence selected from the sequences shown in SEQ ID NO 5, SEQ ID NO 8, SEQ ID NO 11, and SEQ ID NO 17,

(g) an amino acid comprising at least 200 contiguous residues of the sequence shown in SEQ ID NO 20,

(h) an amino acid sequence which is selected from the group consisting of SEQ ID NO 2, SEQ ID NO 5, SEQ ID NO 8, SEQ ID NO 11, SEQ ID NO 14, SEQ ID NO 17, and SEQ ID NO 19.

Moreover, there are provided isolated proteins, wherein said proteins comprise an amino acid sequence selected from the group consisting of:

(a) an amino acid sequence encoded by a nucleic acid sequence comprising at least 150 contiguous nucleotides of the sequence shown in the sequence selected from the group consisting of SEQ ID NO 1, and SEQ ID NO 13.

(b) an amino acid sequence encoded by a nucleic acid sequence comprising at least 500 contiguous nucleotides of the sequence shown in the sequence selected from the group consisting of SEQ ID NO 4, SEQ ID NO 7, SEQ ID NO 10, and SEQ ID NO 16

(c) an amino acid sequence encoded by a nucleic acid sequence comprising at least 700 contiguous nucleotides of the sequence shown in SEQ ID NO 19,

(d) an amino acid sequence encoded by a nucleic acid sequence sequence which is selected from the group consisting of SEQ ID NO 1, SEQ ID NO 4, SEQ ID NO 7, SEQ ID NO 10, SEQ ID NO 13, SEQ ID NO 16, and SEQ ID NO 19,

(e) an amino acid comprising at least 50 contiguous residues of the sequence shown in SEQ ID NO 2, and SEQ ID NO 14,

(f) an amino acid comprising at least 200 contiguous residues of the sequence selected from the sequences shown in SEQ ID NO 5, SEQ ID NO 8, SEQ ID NO 11, and SEQ ID NO 17,

(g) an amino acid comprising at least 300 contiguous residues of the sequence shown in SEQ ID NO 20,

(h) an amino acid sequence which is selected from the group consisting of SEQ ID NO 2, SEQ ID NO 5, SEQ ID NO 8, SEQ ID NO 11, SEQ ID NO 14, SEQ ID NO 17, and SEQ ID NO 19.

Proteins which would result from expression of the nucleic acid molecules herein disclosed are preferred, with the proteins which would result from expression of the exemplified nucleic acid molecules being most preferred (e.g. SEQ ID Nos 2, 5, 8, 11,

14, 17, 20, 23, 26, 29, 32, 35, and 38). Hinge region canine IgG proteins are provided, especially those exemplified (e.g. SEQ ID Nos 2, 8, 14, and 23). Heavy chain canine IgG proteins are also provided, especially those exemplified (e.g. SEQ ID Nos 5, 11, 17, 20, 29, 32, 35, and 38).Light chain sequences are also provided, especially those exemplified (e.g. SEQ ID NO 26). It is understood that proteins which result from expression of allelic variants of the exemplified sequences.

According to the present invention, a canine IgG (heavy and/or light chain) protein of the present invention refers to: a heavy or light chain of canine IgG protein; a heavy or light chain of canine IgG homolog; or a heavy or light chain of canine IgG peptide. Preferably, a heavy chain of canine IgG protein binds to the hinge regions, elicits immune (e.g. antibody) response, induces complement, binds to Fcgamma receptor, etc. In the present invention, a "protein" includes sequences, homologues, fragments (e.g. peptides).

A canine IgG heavy chain protein of the present invention, including a homolog, can be identified in a straight-forward manner by the protein's ability to bind to anti-canine IgG antibodies. Examples of canine IgG (heavy and/or light chain) protein homologs include canine IgG (heavy and/or light chain) proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitoylation, amidation and/or addition of glycerophosphatidyl inositol) such that the homolog is capable of binding to anti-canine IgG antibodies.

In another embodiment, a preferred canine IgG (heavy and/or light chain) protein includes a protein encoded by a nucleic acid molecule that hybridizes under conditions which preferably allow about 35% or less base pair mismatch, more preferably under conditions which allow about 30% or less base pair mismatch, more preferably under conditions which allow about 25% or less base pair mismatch, more preferably under conditions which allow about 20% or less base pair mismatch, more preferably under conditions which allow about 15% or less base pair mismatch, more preferably under conditions which allow about 10% or less base pair mismatch, and even more preferably under conditions which allow about 5% or less base pair mismatch with a nucleic acid

molecule selected from the exemplified nucleic acid molecules.

Another embodiment of the present invention includes a canine IgG (heavy and/or light chain) protein encoded by a nucleic acid molecule selected from the group consisting of: a nucleic acid molecule which hybridizes, in a solution comprising 1X SSC and 0% formamide, at a temperature of about 56°C, to a nucleic acid sequence selected from the exemplified nucleic acid sequences; and a nucleic acid molecule comprising a fragment of any of said nucleic acid molecules.

Yet another preferred canine IgG (heavy and/or light chain) protein of the present invention includes a protein encoded by a nucleic acid molecule which is preferably about at least 45% identical, more preferably about at least 50% identical, more preferably about at least 55% identical, more preferably about at least 60% identical, more preferably about at least 65% identical, more preferably about at least 70% identical, more preferably about at least 75% identical, more preferably about at least 80% identical, more preferably about at least 85% identical, more preferably about at least 90% identical and even more preferably about at least 95% identical, more preferably 100% identical to a presently-disclosed nucleic acid molecule, and/or fragments of such proteins. Percent identity as used herein is determined using the Compare function by maximum matching within the program DNAsis Version 2.1 using default parameters.

Preferred heavy chain canine IgG proteins of the present invention include proteins comprising amino acid sequences that are at least about 40%, particularly at least about 50%, preferably at least about 55%, more preferably at least about 60%, even more preferably at least about 65%, even more preferably at least about 70%, even more preferably at least about 75%, even more preferably at least about 80%, even more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95%, identical to amino acid sequence exemplified herein.

The canine IgG (heavy and/or light chain) protein homologs can be the result of natural allelic variation or natural mutation. Canine IgG protein homologs of the present invention can also be produced using techniques known in the art including, but not limited to, direct modifications to the protein or modifications to the gene encoding the protein using, for example, classic or recombinant nucleic acid techniques to effect

random or targeted mutagenesis.

The minimal size of an IgG protein homolog of the present invention is a size sufficient to be encoded by a nucleic acid molecule capable of forming a stable hybrid (e.g., hybridize under stringent hybridization conditions) with the complementary sequence of a nucleic acid molecule encoding the corresponding natural protein. As such, the size of the nucleic acid molecule encoding such a protein homolog is dependent on nucleic acid composition and percent homology between the nucleic acid molecule and complementary sequence. It should also be noted that the extent of homology required to form a stable hybrid can vary depending on whether the homologous sequences are interspersed throughout the nucleic acid molecules or are clustered (e.g., localized) in distinct regions on the nucleic acid molecules. The minimal size of such nucleic acid molecules is typically at least about 12 to about 15 nucleotides in length if the nucleic acid molecules are GC-rich and at least about 15 to about 17 bases in length if they are AT-rich. As such, the minimal size of a nucleic acid molecule used to encode a heavy chain of canine IgG protein homolog of the present invention is from about 12 to about 18 nucleotides in length. Thus, the minimal size of a heavy chain of canine IgG protein homolog of the present invention is from about 4 to about 6 amino acids in length. There is no limit, other than a practical limit, on the maximal size of such a nucleic acid molecule in that the nucleic acid molecule can include gene, an entire gene, multiple genes, or portions thereof. The preferred size of a protein encoded by a nucleic acid molecule of the present invention depends on whether a full-length, fusion, multivalent, or functional portion of such a protein is desired.

The present invention also includes mimetopes of heavy chain of canine IgG proteins of the present invention. As used herein, a mimetope of a heavy chain of canine IgG protein of the present invention refers to any compound that is able to mimic the activity of such a heavy chain of canine IgG protein (e.g., ability to bind to anti-canine antibodies), often because the mimetope has a structure that mimics heavy chain of canine IgG protein. It is to be noted, however, that the mimetope need not have a structure similar to a heavy chain of canine IgG protein as long as the mimetope functionally mimics the protein. Mimetopes can be, but are not limited to: peptides that have been modified to decrease their susceptibility to degradation; anti-idiotypic and/or

catalytic antibodies, or fragments thereof; non-proteinaceous immunogenic portions of an isolated protein (e.g., carbohydrate structures); synthetic or natural organic or inorganic molecules, including nucleic acids; and/or any other peptidomimetic compounds. Mimetopes of the present invention can be designed using computer-generated structures of heavy chain of canine IgG proteins of the present invention. Mimetopes can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides or other organic molecules, and screening such samples by affinity chromatography techniques using the corresponding binding partner, (e.g., a canine Fcgamma-binding domain or anti-heavy chain of canine IgG antibody). A mimetope can also be obtained by, for example, rational drug design. In a rational drug design procedure, the three-dimensional structure of a compound of the present invention can be analyzed by, for example, nuclear magnetic resonance (NMR) or x-ray crystallography. The three-dimensional structure can then be used to predict structures of potential mimetopes by, for example, computer modeling. The predicted mimetope structures can then be produced by, for example, chemical synthesis, recombinant DNA technology, or by isolating a mimetope from a natural source. Specific examples of heavy chain of canine IgG mimetopes include anti-idiotypic antibodies, oligonucleotides produced using Selex™ technology, peptides identified by random screening of peptide libraries and proteins identified by phage display technology. A preferred mimetope is a peptidomimetic compound that is structurally and/or functionally similar to a heavy chain of canine IgG protein of the present invention, particularly to the FcgammaR-binding domain of heavy chain of canine IgG protein.

As used herein, an isolated canine IL-13Rα protein can be a full-length protein or any homolog of such a protein. An isolated IL-13Rα protein of the present invention, including a homolog, can be identified in a straight-forward manner by the protein's ability to bind IL-13 or bind to an anti-IL-13Rα protein. Examples of protein homologs of the present invention include proteins of the present invention in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitoylation, amidation and/or addition of glycerophosphatidyl inositol) such that the protein homolog binds IL-13 or includes at

least one epitope capable of eliciting an immune response against the parent protein or binding to an antibody directed against the parent protein, where the term parent refers to the longer and/or full-length protein that the homolog is derived from. Minimal size of epitope is about 4-6 amino acids. Minimal size of IL-13 binding domain can be determined by one skilled in the art.

Homologs of proteins of the present invention can be the result of natural allelic variation, including natural mutation. Protein homologs of the present invention can also be produced using techniques known in the art including, but not limited to, direct modifications to the protein and/or modifications to the gene encoding the protein using, for example, classic or recombinant DNA techniques to effect random or targeted mutagenesis.

One embodiment of the present invention is a canine IL-13Rα1 protein that includes amino acid sequence having SEQ ID NO:50. Preferred is a canine IL-13Rα1 protein that is encoded by nucleic acid sequences SEQ ID NO:48, SEQ ID NO:49 and/or SEQ ID NO:52.

In another embodiment of the present invention is a canine IL-13Rα 2 protein that includes at least one of the following amino acid sequences SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:61, SEQ ID NO:66, and SEQ ID NO:69. Preferred is a canine IL-13Rα 2 protein encoded by one or more of the following nucleic acid sequences: the nucleic acid sequence SEQ ID NO:54, SEQ ID NO:57, SEQ ID NO:60, SEQ ID NO:63, SEQ ID NO:65 and/or SEQ ID NO:68.

In one embodiment of the present invention, isolated IL-13Rα proteins are encoded by nucleic acid molecules that hybridize under stringent hybridization conditions to the non-coding strand of nucleic acid molecules encoding canine IL-13Rα proteins. The minimal size of a IL-13R protein of the present invention (4-6 amino acids) is a size sufficient to be encoded by a nucleic acid molecule capable of forming a stable hybrid, i.e., hybridizing under stringent hybridization conditions, with the complementary sequence of a nucleic acid molecule encoding the corresponding natural protein. The size of a nucleic acid molecule (12-17/18 nucleotides) encoding such a protein is dependent on the nucleic acid composition and the percent homology between the IL-13Rα nucleic acid molecule and the complementary nucleic acid sequence. It can easily be understood

that the extent of homology required to form a stable hybrid under stringent conditions can vary depending on whether the homologous sequences are interspersed throughout a given nucleic acid molecule or are clustered, i.e. localized, in distinct regions on a given nucleic acid molecule.

A preferred canine IL-13Rα protein includes a protein encoded by a nucleic acid molecule selected from the group consisting of:(a) an isolated nucleic acid molecule comprising at least 75 contiguous nucleotides identical in sequence to an at least 75 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:48, SEQ ID NO:49 and/or SEQ ID NO:52; and (b) an isolated nucleic acid molecule comprising a nucleic acid sequence that is at least 90% identical in sequence to a nucleic acid sequence to a nucleic acid sequence selected from the group consisting of SEQ ID NO:48, SEQ ID NO:49 and/or SEQ ID NO:52, wherein the percent identity is determined by the DNAsis™ computer program with the gap penalty set at 5, the number of top diagonals set at 5, the fixed gap penalty set at 10, the k-tuple set at 2, the window size set at 10 and the floating gap penalty set at 10.

Another preferred canine protein of the present invention includes a protein encoded by an IL-13Rα1 nucleic acid molecule that is preferably at least 90% identical, more preferably at least 92%, more preferably at least 94%, more preferably at least 96%, and even more preferably at least 98% identical to a nucleic acid molecule having the nucleic acid sequence SEQ ID NO:48, and/or SEQ ID NO:49; wherein the percent identity is determined by the DNAsis™ computer program with the gap penalty set at 5, the number of top diagonals set at 5, the fixed gap penalty set at 10, the k-tuple set at 2, the window size set at 10 and the floating gap penalty set at 10. Also preferred are fragments of such proteins encoded by nucleic acid molecules that are at least about 75 nucleotides, at least about 80 nucleotides, at least about 85 nucleotides, at least about 90 nucleotides, at least about 95 nucleotides, at least about 100 nucleotides, at least about 120 nucleotides, at least about 140 nucleotides, at least about 160 nucleotides, at least about 180 nucleotides, at least about 200 nucleotides, at least about 250 nucleotides, at least about 300 nucleotides, at least about 350 nucleotides, or at least about 400 nucleotides at least about 500 nucleotides, at least about 600 nucleotides, at least about 700 nucleotides, at least about 800 nucleotides, at least about 900 nucleotides, at least

about 1000 nucleotides, at least about 1100 nucleotides, at least about 1300 nucleotides, or at least about 1500 nucleotides.

In one embodiment, a preferred protein of the present invention includes an IL-13Rα1 protein that is preferably at least about 85%, even more preferably at least about 90%, even more preferably at least about 95%, and even more preferably about 100% identical to PCaIL-13Rα1$_{405}$, with amino acid sequence SEQ ID NO:50. Additionally preferred are proteins encoded by allelic variants of a nucleic acid molecule encoding PCaIL-13Rα1$_{405}$. Also preferred are fragments thereof having at least about 45 amino acid residues as well as fragments thereof are at least 50 amino acids, at least 55 amino acids, at least 60 amino acids, at least 75 amino acids, at least 100 amino acids, at least 150 amino acids, at least 200 amino acids, at least 250 amino acids, at least 300 amino acids, at least 350 amino acids, or at least 400 amino acids in length.

A preferred canine IL-13Rα protein includes a protein encoded by a nucleic acid molecule consisting of an IL-13Rα2 nucleic acid molecule selected from the group consisting of: (a) an isolated nucleic acid molecule comprising at least 40 contiguous nucleotides identical in sequence to an at least 40 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:54, SEQ ID NO:57, SEQ ID NO:60, SEQ ID NO:63, SEQ ID NO:65 and/or SEQ ID NO:68; and (b) an isolated nucleic acid molecule comprising a nucleic acid sequence that is at least 80% identical in sequence to a nucleic acid sequence selected from the group consisting of SEQ ID NO:54, SEQ ID NO:57, SEQ ID NO:60, SEQ ID NO:63, SEQ ID NO:65 and/or SEQ ID NO:68, wherein the percent identity is determined by the DNAsis™ computer program with the gap penalty set at 5, the number of top diagonals set at 5, the fixed gap penalty set at 10, the k-tuple set at 2, the window size set at 10 and the floating gap penalty set at 10. In one embodiment, such a nucleic acid molecule does not hybridize under conditions comprising hybridization at 65°C in 0.1 X SSC followed by washing at 65°C in 0.1 X SSC with a nucleic acid sequence selected from the group consisting of SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97 and SEQ ID NO:98. Also preferred are proteins encoded by portions of such nucleic acid molecules that are at least about 40 nucleotides, at least 50 nucleotides, at least 55 nucleotides, at least 60 nucleotides, at least 70 nucleotides, at least 80 nucleotides, at least 90 nucleotides, at least 100 nucleotides, at

least 150 nucleotides, at least 200 nucleotides, at least 300 nucleotides, at least 400 nucleotides, at least 500 nucleotides, at least 600 nucleotides, or at least 700 nucleotides at least 800 nucleotides, at least 900 nucleotides, at least 1000 nucleotides, at least 1100 nucleotides, at least 1200 nucleotides, at least 1300 nucleotides, at least 1400 nucleotides in length.

Another preferred canine protein of the present invention includes a protein encoded by an IL-13Rα2 nucleic acid molecule that is preferably at least 80% identical, more preferably at least 85% identical, more preferably at least 90% identical, more preferably at least 95% identical to a nucleic acid molecule having the nucleic acid sequence SEQ ID NO:54, SEQ ID NO:57, SEQ ID NO:60, SEQ ID NO:63, SEQ ID NO:65 and/or SEQ ID NO:68; also preferred are fragments, i.e. portions, of such proteins encoded by nucleic acid molecules that are at least about 40 nucleotides, wherein the percent identity is determined by the DNAsis™ computer program with the gap penalty set at 5, the number of top diagonals set at 5, the fixed gap penalty set at 10, the k-tuple set at 2, the window size set at 10 and the floating gap penalty set at 10. In one embodiment, such a nucleic acid molecule does not hybridize under conditions comprising hybridization at 65°C in 0.1 X SSC followed by washing at 65°C in 0.1 X SSC with a nucleic acid sequence selected from the group consisting of SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97 and SEQ ID NO:98.

Another preferred protein of the present invention includes an IL-13Rα2 protein that is preferably at least 70% identical, more preferably at least 75% identical, more preferably at least 80% identical, more preferably at least 85% , more preferably at least 90% , more preferably at least 95% , and even more preferably about 100% identical to PCaIL-13Rα2_{145}, PCaIL-13Rα2_{255}, PCaIL-13Rα2_{386}, PCaIL-13Rα2_{365}, and/or PCaIL-13Rα2_{318}. Additionally preferred are proteins encoded by allelic variants of a nucleic acid molecule encoding proteins PCaIL-13Rα2_{145}, PCaIL-13Rα2_{255}, PCaIL-13Rα2_{386}, PCaIL-13Rα2_{365}, and/or PCaIL-13Rα2_{318}. Also preferred are fragments thereof having at least about 40, at least about 45, at least about 50, at least about 55, at least about 60, at least about 65, at least about 70, at least about 75, at least about 80, at least about 85, at least about 90, at least about 95, at least about 100, at least about 120, at least about 140, at least about 150, at least about 175, at least about 200, at least about 225, at least about

250, at least about 275, at least about 300, at least about 325 or at least about 350 amino acid residues.

In one embodiment of the present invention, canine IL-13Rα2 proteins comprise amino acid sequence SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:61, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:72, SEQ ID NO:75, SEQ ID NO:78 and/or SEQ ID NO 81. Such proteins include, but are not limited to, the proteins consisting of the cited amino acid sequences, fusion proteins and multivalent proteins, and proteins encoded by allelic variants of nucleic acid molecules encoding proteins having amino acid sequence SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:61, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:72, SEQ ID NO:75, SEQ ID NO:78 and/or SEQ ID NO 81.

One embodiment of a heavy chain of canine IgG protein of the present invention is a fusion protein that includes a heavy chain of canine IgG protein domain attached to one or more fusion segments. Suitable fusion segments for use with the present invention include, but are not limited to, segments that can: enhance a protein's stability; act as an immunopotentiator to enhance an immune response; act as an suppressor of immune response and/or assist purification of a heavy chain of canine IgG protein (e.g., by affinity chromatography). A suitable fusion segment can be a domain of any size that has the desired function (e.g., imparts increased stability, imparts increased immunogenicity to a protein, and/or simplifies purification of a protein). Fusion segments can be joined to amino and/or carboxyl termini of heavy chain of canine IgG-containing domain of the protein and can be susceptible to cleavage in order to enable straight-forward recovery of a heavy chain of canine IgG protein. Fusion proteins are preferably produced by culturing a recombinant cell transformed with a fusion nucleic acid molecule that encodes a protein including the fusion segment attached to either the carboxyl and/or amino terminal end of a heavy chain of canine IgG-containing domain. Preferred fusion segments include a metal binding domain (e.g., a poly-histidine segment); an immunoglobulin binding domain (e.g., Protein A; Protein G; T cell; B cell; Fc receptor or complement protein antibody-binding domains); a sugar binding domain (e.g., a maltose binding domain); a "tag" domain (e.g., at least a portion of ß-galactosidase, a strep tag peptide, other domains that can be purified using compounds that bind to the domain, such as monoclonal antibodies); and/or a linker and enzyme domain (e.g., alkaline

phosphatase domain connected to a heavy chain of canine IgG protein by a linker). More preferred fusion segments include metal binding domains, such as a poly-histidine segment; a maltose binding domain; a strep tag peptide, such as that available from Biometra in Tampa, FL; and a phage T7 S10 peptide.

In general, the present invention also comprises chimera of the following types: immunotoxins (the present proteins comprising a toxin, and directed to a particular target, either an IgG binding site, or a binding site of a variable region fused with an IgG protein herein), scavenger molecules (an IgG/variable region chimera designed so as to "clean up" unwanted compounds in the cellular milleu, by virtue of their ability to bind to them), drug delivery vehicles (IgG with a drug attached, and directed either to IgG binding site or to a site of choice), and molecules with increased half-life.

A heavy chain of canine IgG molecule of the present invention can also include chimeric molecules comprising canine IgG molecule and a second molecule that enables the chimeric molecule to be bound to a substrate. An example of a suitable second molecule includes an immunoglobulin molecule or another ligand that has a suitable binding partner that can be immobilized on a substrate, e.g., biotin and avidin, or a metal-binding protein and a metal (e.g., His), or a sugar-binding protein and a sugar (e.g., maltose).

Chimeric immunoglobulin molecules are also included in the present invention. Specifically, a chimeric immunoglobulin molecule which contains a portion from a heavy chain of canine IgG and a portion that is not canine is contemplated. The non-canine portion is preferably the antigen binding site of the chimeric immunoglobulin. A chimeric molecule ideally contains only those portions of the non-canine variable region that bind to antigen, with the remainder of the immunoglobulin comprising canine sequence.

One embodiment of a canine IL-13Rα protein of the present invention is a fusion protein that includes a canine IL-13Rα2 protein-containing domain attached to one or more fusion segments. Suitable fusion segments for use with the present invention include, but are not limited to, segments that can: enhance a protein's stability; act as an immunopotentiator to enhance an immune response against canine IL-13Rα protein; and/or assist in purification of a canine IL-13Rα protein, e.g., by affinity

chromatography. A suitable fusion segment can be a domain of any size that has the desired function, e.g., imparts increased stability, imparts increased immunogenicity to a protein, and/or simplifies purification of a protein. Fusion segments can be joined to amino and/or carboxyl termini of the IL-13Rα containing domain of the protein and can be susceptible to cleavage in order to enable straight-forward recovery of a IL-13Rα protein. Fusion proteins are preferably produced by culturing a recombinant cell transformed with a fusion, or chimeric nucleic acid molecule that encodes a protein including the fusion segment attached to either the carboxyl and/or amino terminal end of a IL-13Rα containing domain. Preferred fusion segments include an immunoglobulin domains, a metal binding domain, e.g., a poly-histidine segment; an immunoglobulin binding domain, e.g., Protein A, Protein G, T cell, B cell, Fc receptor or complement protein antibody-binding domains; a sugar binding domain, e.g., a maltose binding domain; and/or a tag domain, e.g., at least a portion of β-galactosidase, a strep tag peptide, a T7 tag peptide, a Flag™ peptide, or other domains that can be purified using compounds that bind to the domain, such as monoclonal antibodies. More preferred fusion segments include immunological domains such as Fcγ, Fcε, Fcα, Fcμ , or Fcδ domains; metal binding domains, such as a poly-histidine segment; a maltose binding domain; a strep tag peptide, such as that available from Biometra in Tampa, FL; and an S10 peptide. One embodiment of a fusion protein is a chimera of IL-13Ra domain with a functional moiety. This moiety can, for example, have the function of allowing oligomerization of the chimeric IL-13Ra proteins.

In one embodiment, a canine IL-13Rα protein of the present invention is a fusion protein that includes a carrier protein domain linked to an IL-13Rα domain such that either the carrier protein domain or the IL-13Rα domain can be the carboxyl terminal domain. Such a protein is preferably encoded by a chimeric nucleic acid molecule that includes a carrier protein encoding domain and an IL-13Rα encoding domain. A fusion protein of the present invention can also include a linker (i.e. a stretch of one or more additional amino acids) preferably located between the carrier protein domain and IL-13Rα domain. As used herein, a carrier protein domain has a meaning similar to a fusion segment. A preferred carrier protein domain is an immunoglobulin IgFc region,

preferably a canine IFC region. In one embodiment, a canine IgE region, and preferably at least a portion of a canine gamma chain is preferred. Examples of canine gamma chains are disclosed herein. A fusion protein of the present invention can include any IL-13Rα protein of the present invention. Preferably the IL-13Rα domain binds IL-13. More preferred is a IL-13Rα2 protein of the present invention. Similarly, a chimeric nucleic acid molecule encoding a fusion protein of the present invention can include any IL-13Rα nucleic acid molecule of the present invention. Preferred is a IL-13Rα nucleic acid molecule that binds IL-13. More preferred is a IL-13Rα2 nucleic acid molecule of the present invention. A particularly preferred fusion protein of the present invention includes one or more of the following amino acid sequences: SEQ ID NO: 72, SEQ ID NO:75, SEQ ID NO:78 and/or SEQ ID NO:81. Production of such fusion proteins is described in the Examples. A preferred chimeric nucleic acid molecule of the present invention encodes one of such fusion proteins.

A particularly preferred chimera nucleic acid molecule of the present invention includes one or more of the following nucleic acid sequences: the nucleic acid sequence SEQ ID NO:71, SEQ ID NO:74, SEQ ID NO:77 and/or SEQ ID NO:80, and/or the respective complements of these nucleic acid sequences, i.e. SEQ ID NO:73, SEQ ID NO:76, SEQ ID NO:79 and/or SEQ ID NO:82, respectively. These nucleic acid sequences which contain canine IL-13Rα2 nucleic acid molecules linked to canine IgE-Fc nucleic acid molecules are further described herein. For example, SEQ ID NO:77 represents the deduced sequence of the coding strand of canine chimera nucleic acid molecule nCaIL-13Rα2-Fc-B9, the cloning of which is disclosed in the examples. The complement of SEQ ID NO:77, represented herein by SEQ ID NO:79, refers to the nucleic acid sequence of the strand complementary to the strand having SEQ ID NO:77, which can be easily be determined by those skilled in the art. The nucleic acid sequences of the coding strand and complementary strand of nCa IL-13Rα 2-Fc-B9 are represented herein as SEQ ID NO:77 and SEQ ID NO:79, respectively. Translation of SEQ ID NO:77 indicates that nucleic acid molecule nCa IL-13Rα 2-Fc-B9 encodes a fusion protein of about 563 amino acids, denoted herein as PCa IL-13Rα 2-Fc-B9, the amino acid sequence of which is presented in SEQ ID NO:78, assuming an open reading frame having an initiation codon spanning from nucleotide 1 through nucleotide 3 of SEQ ID

NO:77 and a stop codon spanning from nucleotide 1690 through nucleotide 1692 of SEQ ID NO:77. Other chimeric nucleic acid molecules are discussed in more detail in the examples.

A preferred fusion protein is encoded by a chimeric nucleic acid molecule that includes:(a) a nucleic acid sequence encoding a carrier protein domain; and (b) an nucleic acid molecule encoding an IL-13Rα2 protein domain selected from the group consisting of:(i) an isolated nucleic acid molecule comprising at least 40 contiguous nucleotides identical in sequence to an at least 40 contiguous nucleotide region of a nucleic acid sequence selected from the group consisting of SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64,SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68 and SEQ ID NO:70; and (ii) an isolated nucleic acid molecule comprising a nucleic acid sequence that is at least 80% identical in sequence to a nucleic acid sequence selected from the group consisting of SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64,SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68 and SEQ ID NO:70, wherein the percent identity is determined by the DNAsis™ computer program with the gap penalty set at 5, the number of top diagonals set at 5, the fixed gap penalty set at 10, the k-tuple set at 2, the window size set at 10 and the floating gap penalty set at 10. In one embodiment, such a nucleic acid molecule does not hybridize under conditions comprising hybridization at 65°C in 0.1 X SSC followed by washing at 65°C in 0.1 X SSC with a nucleic acid sequence selected from the group consisting of SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97 and SEQ ID NO:98.

Yet another preferred canine fusion protein of the present invention includes a protein that is encoded by chimeric nucleic acid molecule with an IL-13α2 encoding domain that is preferably at least 80% identical, more preferably at least 85% identical, more preferably at least 90% identical, more preferably at least 95% identical to a nucleic acid molecule having the nucleic acid sequence SEQ ID NO:54, SEQ ID NO:57, SEQ ID NO:60, SEQ ID NO:63, SEQ ID NO:65 and/or SEQ ID NO:68; also preferred are fragments, i.e. portions, of such proteins encoded by nucleic acid molecules that are at least about 40 nucleotides, wherein the percent identity is determined by the DNAsis™ computer program with the gap penalty set at 5, the number of top diagonals set at 5, the

fixed gap penalty set at 10, the k-tuple set at 2, the window size set at 10 and the floating gap penalty set at 10. In one embodiment, such a nucleic acid molecule does not hybridize under conditions comprising hybridization at 65°C in 0.1 X SSC followed by washing at 65°C in 0.1 X SSC with a nucleic acid sequence selected from the group consisting of SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97 and SEQ ID NO:98.

Another preferred protein of the present invention includes fusion protein comprising a carrier protein domain and a IL-13Rα2 protein domain that is preferably at least 70% identical, more preferably at least 75% identical, more preferably at least 80% identical, more preferably at least 85% , more preferably at least 90% , more preferably at least 95% , and even more preferably about 100% identical to PCaIL-13Rα2$_{145}$, PCaIL-13Rα2$_{255}$, PCaIL-13Rα2$_{386}$, PCaIL-13Rα2$_{365}$, and/or PCaIL-13Rα2$_{318}$. Additionally preferred are proteins encoded by allelic variants of a nucleic acid molecule encoding proteins PCaIL-13Rα2$_{145}$, PCaIL-13Rα2$_{255}$, PCaIL-13Rα2$_{386}$, PCaIL-13Rα2$_{365}$, and/or PCaIL-13Rα2$_{318}$. Also preferred are fragments thereof having at least about 40 amino acid residues.

One embodiment includes a canine IL-13Rα protein that is capable of binding IL-13. For a protein to be capable of binding to its ligand, in this case IL-13, the protein must have a functional binding domain. A functional binding domain is at least the smallest piece, or fragment, of the protein that is necessary to allow binding to a IL-13. For example, a functional binding domain also includes proteins that are larger than the smallest fragment necessary to allow binding to IL-13 or receptor. A preferred canine IL-13Rα protein comprises of an amino acid sequence selected from the group consisting of SEQ ID NO:50, SEQ ID NO:55, SEQ ID NO:58,SEQ ID NO:61, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:72, SEQ ID NO:75, SEQ ID NO:78 and/or SEQ ID NO:81 and/or a fragment thereof, such that the fragment is capable of binding to canine IL-13; and a protein encoded by an allelic variant of a nucleic acid molecule which encodes any such protein. One skilled in the art can determine in a straight-forward manner whether an IL-13Rα protein binds IL-13. Examples of such methods include, determining the ability of an IL-13Rα protein to form a complex with IL-13 or determining the ability of an IL-13Rα protein to inhibit IL-13 stimulated Td-1 cell proliferation. Examples of such

methods are disclosed herein.

One of skill in the art will understand that a DNA or protein fragment of the present invention is an example of a homolog that includes a portion of a larger nucleic acid molecule or protein, respectively, of the present invention. One of skill in the art will also understand that fragments including one or more of the functional domains of IL-13Rα can vary and extend beyond those particular nucleic acid or amino acid regions defined herein. Such active domains can vary in length by 1 amino acid to about 200 amino acids. Nucleic acids or amino acids essential to an active domain can be identified using standard protein or DNA binding assays known to those of skill in the art to determine the ability of an active domain to bind to its ligand(s), e.g. IL-13, or to its receptor(s), e.g. IL-13Rα.

Also provided in the present invention are recombinant cells comprising the fusion proteins describe herein.

A variety of procedures known in the art may be used to molecularly clone canine IgG (heavy and/or light chain) nucleic acid molecules and/or canine IL-13Rα nucleic acid molecules of the present invention. These methods include, but are not limited to, direct functional expression of a canine IgG (heavy and/or light chain) nucleic acid molecules and/or canine IL-13Rα nucleic acid molecules following construction of the heavy chain of a canine IgG-containing and/or canine IL-13Rα containing cDNA or genomic DNA library in an appropriate expression vector system. Another method is to screen a canine IgG (heavy and/or light chain)-containing and/or a canine IL-13Rα-containing cDNA or genomic DNA library constructed in a bacteriophage or plasmid shuttle vector with a labeled oligonucleotide probe designed from the amino acid sequence of a canine IgG (heavy and/or light chain) protein and/or a canine IL-13Rα protein of the present invention. An additional method consists of screening a canine IgG (heavy and/or light chain)-containing and/or a canine IL-13Rα-containing cDNA or genomic DNA library constructed in a bacteriophage or plasmid shuttle vector with a canine IgG nucleic acid molecule and/or canine IL-13Rα nucleic acid molecule of the present invention. Such a nucleic acid molecule can be is obtained by PCR amplification of canine IgG (heavy and/or light chain) nucleic acid molecule fragments and/or canine IL-13Rα nucleic acid molecule fragments through the design of degenerate

oligonucleotide primers from the amino acid sequence of canine IgG (heavy and/or light chain) and/or canine IL-13Rα or of primers from canine IgG and/or canine IL-13Rα nucleic acid molecules.

The translation of the RNA into a protein will result in the production of at least a portion of canine IgG (heavy and/or light chain) and/or canine IL-13Rα protein, or fusions thereof, which can be identified, for example, by the activity of a canine IgG (heavy and/or light chain) and/or canine IL-13Rα protein or by immunological reactivity with an anti-canine IgG (heavy and/or light chain) and/or anti-canine IL-13R antibody. In this method, pools of mRNA isolated from canine IgG (heavy and/or light chain) and/or canine IL-13Rα protein-producing cells can be analyzed for the presence of an RNA which encodes at least a portion of a canine IgG (heavy and/or light chain) and/or canine IL-13Rα protein. Further fractionation of the RNA pool can be done to purify canine IgG (heavy and/or light chain) and/or canine IL-13Rα RNA from non-canine IgG (heavy and/or light chain) and/or canine IL-13Rα RNA. Protein produced by isolataion of RNA can be analyzed to provide amino acid sequences which in turn are used to provide primers for production of canine IgG (heavy and/or light chain) and/or canine IL-13Rα cDNA, or the RNA used for translation can be analyzed to provide nucleotide sequences encoding canine IgG (heavy and/or light chain) and/or canine IL-13Rα and produce probes for the production of canine IgG (heavy and/or light chain) and/or canine IL-13Rα cDNA. These methods are known in the art and can be found in, for example, Sambrook, J., Fritsch, E. F., Maniatis, T. in *Molecular Cloning: A Laboratory Manual, Second Edition*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 1989.

Other types of libraries, as well as libraries constructed from other cells or cell types, may be useful for isolating canine IgG (heavy and/or light chain) and/or canine IL-13Rα-encoding DNA. Other types of libraries include, but are not limited to, cDNA libraries derived from other canines or cell lines derived from other canines, and genomic DNA libraries. Preparation of cDNA libraries can be performed by standard techniques. Well known cDNA library construction techniques can be found in, for example, Sambrook, J., et al., *ibid.*

Nucleic acid molecules encoding canine IgGs and/or canine IL-13Rα proteins can also be isolated from a suitable genomic DNA library. Construction of genomic DNA

libraries can be performed by standard techniques. Well known genomic DNA library construction techniques can be found in Sambrook, J., et al., *ibid.*

In order to clone a canine IgG (heavy and/or light chain) and/or canine IL-13Rα nucleic acid molecule by the above methods, knowledge of the amino acid sequence of said molecules may be necessary. One may either use the sequences herein exemplified or purify canine IgG (heavy and/or light chain) and/or canine IL-13Rα protein and sequence a portion of the protein by manual or automated sequencing. It is not necessary to determine the entire amino acid sequence, because the linear sequence of two regions of 6 to 8 amino acids from the protein can be determined and used to produce primers for PCR amplification of a canine IgG (heavy and/or light chain) and/or canine IL-13Rα nucleic acid molecule.

Once suitable amino acid sequences have been identified, DNA sequences capable of encoding such amino acid sequences are synthesized. Because the genetic code is degenerate, more than one codon may be used to encode a particular amino acid, and therefore, the amino acid sequence can be encoded by any of a set of similar DNA oligonucleotides. Only one member of the set will be identical to a canine IgG (heavy and/or light chain) and/or canine IL-13Rα sequence but will be capable of hybridizing to such nucleic acid molecules even in the presence of DNA oligonucleotides with mismatches under appropriate conditions.

Isolated canine IgG (heavy and/or light chain) proteins and canine Il-13Rα proteins of the present invention can be produced in a variety of ways, including production and recovery of natural proteins, production and recovery of recombinant proteins, and chemical synthesis of the proteins. In one embodiment, an isolated protein of the present invention is produced by culturing a cell capable of expressing the protein under conditions effective to produce the protein, and recovering the protein. A preferred cell to culture is a recombinant cell of the present invention. Effective culture conditions include, but are not limited to, effective media, bioreactor, temperature, pH and oxygen conditions that permit protein production. An effective medium refers to any medium in which a cell is cultured to produce a canine IgG (heavy and/or light chain) and/or canineIL-13Rα protein of the present invention. Such a medium typically comprises an aqueous medium having assimilable carbon, nitrogen and phosphate sources, and

appropriate salts, minerals, metals and other nutrients, such as vitamins. Cells of the present invention can be cultured in conventional fermentation bioreactors, shake flasks, test tubes, microtiter dishes, and petri plates. Culturing can be carried out at a temperature, pH and oxygen content appropriate for a recombinant cell. Such culturing conditions are within the expertise of one of ordinary skill in the art.

Depending on the vector and host system used for production, resultant proteins of the present invention may either remain within the recombinant cell; be secreted into the fermentation medium; be secreted into a space between two cellular membranes, such as the periplasmic space in *E. coli*; or be retained on the outer surface of a cell or viral membrane. The phrase "recovering the protein", as well as similar phrases, refers to collecting the whole fermentation medium containing the protein and need not imply additional steps of separation or purification. Proteins of the present invention can be purified using a variety of standard protein purification techniques, such as, but not limited to, affinity chromatography, ion exchange chromatography, filtration, electrophoresis, hydrophobic interaction chromatography, gel filtration chromatography, reverse phase chromatography, concanavalin A chromatography, chromatofocusing and differential solubilization. Proteins of the present invention are preferably retrieved in "substantially pure" form. As used herein, "substantially pure" refers to a purity that allows for the effective use of the protein as a therapeutic composition or diagnostic. A therapeutic composition for animals, for example, should exhibit few impurities.

In addition, the recombinant canine IgG (heavy and/or light chain) and/or canine IL-13Rα proteins can be separated from other cellular proteins by use of an immunoaffinity column made using a substance that selectively binds to said proteins, such as a monoclonal or polyclonal antibodies that selectively bind the full length nascent canine IgG (heavy and/or light chain) and/or canine IL-13Rα proteins or polypeptide fragments of such proteins, an Fcgamma receptor protein, Protein A, etc.

Antibodies selective for a protein of the present invention, isolated cells cell comprising at least one protein of the present invention, and isolated fusion protein comprising at least one protein of the present invention are also within the scope of the present invention.

The present invention also includes isolated (e.g., removed from their natural

milieu) antibodies that selectively bind to a the canine IgG (heavy and/or light chain) protein of the present invention or a mimetope thereof (e.g., anti-heavy chain antibodies). As used herein, the term "selectively binds to" a the canine IgG (heavy and/or light chain) protein refers to the ability of antibodies of the present invention to preferentially bind to specified proteins and mimetopes thereof of the present invention. Binding can be measured using a variety of methods standard in the art including enzyme immunoassays (e.g., ELISA), immunoblot assays, etc.; see, for example, Sambrook et al., *ibid.* An anti-the canine IgG (heavy and/or light chain) antibody preferably selectively binds to a the canine IgG (heavy and/or light chain) protein in such a way as to reduce the activity of that protein.

In particular, there are provided antibodies directed to the heavy chain of canine IgG. Preferred are antibodies selective for the hinge region of the heavy chain of canine IgG. In one preferred embodiment, there are provided antibodies selective for a protein selected from the group consisting of: the exemplified hinge region or heavy chain canine IgG proteins exemplified. These antibodies may be admixed or conjugated with additional materials, such as cytotic agents or other antibody fragments, including IgG fragments.

Isolated anti-canine IgG antibodies of the present invention can include antibodies in a bodily fluid (such as, but not limited to, serum), or antibodies that have been purified to varying degrees. Antibodies of the present invention can be polyclonal or monoclonal. Functional equivalents of such antibodies, such as antibody fragments and genetically-engineered antibodies (including single chain antibodies or chimeric antibodies that can bind to more than one epitope) are also included in the present invention.

A preferred method to produce anti-canine IgG antibodies of the present invention includes (a) administering to an animal an effective amount of a protein or mimetope thereof of the present invention to produce the antibodies and (b) recovering the antibodies. Anti-canine IgG antibodies of the present invention can also be produced recombinantly using techniques as heretofore disclosed to produce the heavy chain of canine IgG proteins of the present invention. Antibodies raised against defined proteins or mimetopes can be advantageous because such antibodies are not substantially contaminated with antibodies against other substances that might otherwise cause

interference in a diagnostic assay or side effects if used in a therapeutic composition.

Anti-canine IgG antibodies of the present invention have a variety of potential uses that are within the scope of the present invention. For example, such antibodies can be used (a) as tools to detect total IgG and/or a specific IgG subclass, (b) to screen expression libraries, (c) to reduce IgG function, and/or (d) to recover desired proteins of the present invention from a mixture of proteins and other contaminants.

The present invention also includes isolated, i.e., removed from their natural milieu, antibodies that selectively bind to a canine IL-13Ra protein of the present invention or a mimetope thereof, e.g., anti-canine IL-13Ra antibodies. As used herein, the term selectively binds to an IL-13Ra protein refers to the ability of antibodies of the present invention to preferentially bind to specified proteins and mimetopes thereof of the present invention. Binding can be measured using a variety of methods standard in the art including enzyme immunoassays, e.g., ELISA, immunoblot assays, etc.; see, for example, Sambrook et al., *ibid.*, and Harlow, et al., 1988, *Antibodies, a Laboratory Manual*, Cold Spring Harbor Labs Press; Harlow et al., *ibid.*, is incorporated herein by reference in its entirety. An anti-IL-13Ra antibody of the present invention preferably selectively binds to a canine IL-13Ra protein in such a way as to inhibit the function of that protein.

Isolated antibodies of the present invention can include antibodies in serum, or antibodies that have been purified to varying degrees. Antibodies of the present invention can be polyclonal or monoclonal, or can be functional equivalents such as antibody fragments and genetically-engineered antibodies, including single chain antibodies or chimeric antibodies that can bind to one or more epitopes.

A preferred method to produce antibodies of the present invention includes (a) administering to an animal an effective amount of a protein, peptide or mimetope of the present invention to produce the antibodies and (b) recovering the antibodies. In another method, antibodies of the present invention are produced recombinantly using techniques as heretofore disclosed to produce IL-13Rα proteins of the present invention. Antibodies raised against defined proteins or mimetopes can be advantageous because such antibodies are not substantially contaminated with antibodies against other substances that might otherwise cause interference in a diagnostic assay or side effects if used in a

therapeutic composition.

Antibodies of the present invention have a variety of potential uses that are within the scope of the present invention. For example, such antibodies can be used (a) to evaluate the immune status in canids with diseases such as allergy, cancer and pathogen infections. Furthermore, antibodies of the present invention can be used to target cytotoxic agents to canine IL-13Rα and cells containing canine IL-13Rα on the cell surface. Targeting can be accomplished by conjugating, i.e., stably joining, such antibodies to the cytotoxic agents using techniques known to those skilled in the art. Suitable cytotoxic agents are known to those skilled in the art. Furthermore, antibodies of the present invention can be used to detect IL-13Rα in a putative IL-13Rα containing biological sample, by contacting the putative IL-13Rα containing biological sample with anti-IL-13Ra antibodies under conditions suitable for formation of a IL-13Rα-antibody complex, and then detecting said complex. Methods to detect said method are known to those skilled in the art and are contained herein.

In general, the present invention provides methods to detect any of the following: canine IgG, cDNA encoding IgG (especially for research purposes), mRNA encoding IgG (for research as well as diagnostic detection purposes), etc. Means for detection may include: antibodies directed to canine IgG protein, including polyclonal or monoclonal antibodies directed to specific regions or specific subclasses, probes identified as hybridizable to the cDNA or mRNA, primers useful for amplifying canine IgG nucleic acid. The following patents describe such procedures, and are hereby incorporated by reference in this patent application: General characteristics of diagnostic reagents and methods to produce and use such diagnostic reagents are disclosed, for example, in United States Patent No. 5,958,880, issued September 28, 1999, by Frank et al.; PCT International Publication No. WO 99/54349, published October 28, 1999, by McCall et al.; PCT Application Serial No. PCT/US99/21428, filed September 18, 1999, by Jensen; United States Patent Application Serial No. 09/479,614, entitled "FELINE IMMUNOGLOBULIN E MOLECULES AND RELATED METHODS", filed January 7, 2000, by McCall et al.; United States Provisional Patent Application Serial No. 60/195,659, entitled CANINE IL-13 RECEPTORS, PROTEINS, NUCLEIC ACIDS AND USES THEREOF", filed April 7, 2000, by Tang; each of these references is

incorporated by reference herein in its entirety; furthermore, the disclosed reagents and methods are incorporated by reference herein in their entireties. It is to be noted that although the reagents and methods disclosed in each of the citations do not relate to the canine IgG proteins, nucleic acid molecules, antibodies and inhibitors of the present invention per se, the disclosed reagents and methods are applicable by those skilled in the art to diagnostic reagents, kits and detection methods of the present invention. General characteristics of therapeutic compositions and methods to produce and use such therapeutic compositions are disclosed, for example, in United States Patent No. 5,958,880, issued September 28, 1999, by Frank et al., and PCT International Publication No. WO 99/54349, published October 28, 1999, by McCall et al., both of which are incorporated by reference herein in their entirety. It is to be noted that although the compositions and methods disclosed in each of the citations do not relate to the canine IgG proteins, nucleic acid molecules, antibodies and inhibitors of the present invention per se, they are applicable by those skilled in the art to therapeutic compositions and methods of the present invention.

In other embodiments, there are provided methods to detect IgG nucleic acid comprising (a) contacting an isolated the canine IgG (heavy and/or light chain) nucleic acid molecule of the present invention with a putative IgG nucleic acid-containing composition under conditions suitable for formation of a canine IgG nucleic acid moleculeIgG nucleic acid complex, and (b) detecting the presence of IgG nucleic acid by detecting the canine IgG nucleic acid molecule IgG nucleic acid complex.

As used herein, the term "contacting" refers to combining or mixing ingredients, as all of those terms are known in the art. "Formation of a complex" refers to the ability of the molecules to form a stable complex that can be measured (e.g., detected). Binding is effected under conditions suitable to form a complex; such conditions (e.g., appropriate concentrations, buffers, temperatures, reaction times) as well as methods to optimize such conditions are known to those skilled in the art, and examples are disclosed herein. Examples of complex formation conditions are also disclosed in, for example, in Sambrook et al., ibid.

In one embodiment, a test compound of the present method includes a biological sample from an animal. A suitable biological sample includes, but is not limited to, a

bodily fluid composition or a cellular composition. A bodily fluid refers to any fluid that can be collected (e.g., obtained) from an animal, examples of which include, but are not limited to, blood, serum, plasma, urine, tears, aqueous humor, cerebrospinal fluid (CSF), saliva, lymph, nasal secretions, traceobronchial aspirations, intestinal secretions, colostrum, milk and feces. Such a composition of the present method can, but need not be, pretreated to remove at least some of the non-IgG isotypes of immunoglobulin and/or other proteins, such as albumin, present in the fluid. Such removal can include, but is not limited to, contacting the bodily fluid with a material, such as Protein G, to remove IgG antibodies and/or affinity purifying IgG antibodies from other components of the body fluid by exposing the fluid to, for example, Concanavalin A. In another embodiment, a composition includes collected bodily fluid that is pretreated to concentrate immunoglobulin contained in the fluid. For example, immunoglobulin contained in a bodily fluid can be precipitated from other proteins using appropriate concentrations of ammonium sulfate. A preferred composition of the present method is serum.

For protein and peptides, complex can be detected in a variety of ways including, but not limited to use of one or more of the following assays: an enzyme-linked immunoassay, a radioimmunoassay, a fluorescence immunoassay, a luminescence assay (e.g.a chemiluminescent assay or a bioluminescent assay), a lateral flow assay, an agglutination assay, a flow-through assay, a particulate-based assay (e.g., using particulates such as, but not limited to, magnetic particles or plastic polymers, such as latex or polystyrene beads), an immunoprecipitation assay, an electonic sensory assay, a BioCore™ assay (e.g., using colloidal gold) and an immunoblotting assay (e.g., a western blot). Such assays are well known to those skilled in the art. Assays can be used to give qualitative or quantitative results depending on how they are used. Some assays, such as agglutination, particulate separation, and immunoprecipitation, can be observed visually (e.g., either by eye or by a machines, such as a densitometer or spectrophotometer) without the need for a detectable marker. Examples of detectable markers include, but are not limited to, a metal-binding label, a physical label, and electronic labela radioactive label, an enzyme, a fluorescent label, a chemiluminescent label, a chromophoric label or a ligand. A ligand refers to a molecule that binds selectively to another molecule. Preferred detectable markers include, but are not limited to,

fluorescein, a radioisotope, a phosphatase (e.g., alkaline phosphatase), biotin, avidin, a peroxidase (e.g., horseradish peroxidase) and biotin-related compounds or avidin-related compounds (e.g., streptavidin or ImmunoPure® NeutrAvidin available from Pierce, Rockford, IL). According to the present invention, a detectable marker can be connected to a the heavy chain of canine IgG molecule using, for example, chemical conjugation or recombinant DNA technology (e.g., connection of a fusion segment such as that described herein for a metal binding domain; an immunoglobulin binding; a sugar binding domain; and a "tag" domain). Preferably a carbohydrate group of the heavy chain of canine IgG molecule is chemically conjugated to biotin.

In one embodiment a complex can be formed and detected in solution. In another embodiment, a complex can be formed in which one or more members of the complex are immobilized on (e.g., coated onto) a substrate. Immobilization techniques are known to those skilled in the art. Suitable substrate materials include, but are not limited to, plastic, glass, gel, celluloid, paper, PVDF (poly-vinylidene-fluoride), nylon, nitrocellulose, and particulate materials such as latex, polystyrene, nylon, nitrocellulose, agarose and magnetic resin. Suitable shapes for substrate material include, but are not limited to, a well (e.g., microtiter dish well), a plate, a dipstick, a bead, a lateral flow apparatus, a membrane, a filter, a tube, a dish, a celluloid-type matrix, a magnetic particle, and other particulates. A particularly preferred substrate comprises an ELISA plate, a dipstick, a radioimmunoassay plate, agarose beads, plastic beads, latex beads, immunoblot membranes and immunoblot papers. In one embodiment, a substrate, such as a particulate, can include a detectable marker.

A preferred method to detect the heavy chain of canine IgG molecules of the present invention is an immunosorbent assay. An immunoabsorbent assay of the present invention comprises a capture molecule and an indicator molecule. A capture molecule of the present invention binds to an IgG in such a manner that the IgG is immobilized to a substrate. As such, a capture molecule is preferably immobilized to a substrate of the present invention prior to exposure of the capture molecule to a putative IgG-containing composition. An indicator molecule of the present invention detects the presence of an IgG bound to a capture molecule. As such, an indicator molecule preferably is not immobilized to the same substrate as a capture molecule prior to exposure of the capture

molecule to a putative IgG-containing composition.

Both a capture molecule and an indicator molecule of the present invention are capable of binding to an IgG. Preferably, a capture molecule binds to a different region of an IgG than an indicator molecule, thereby allowing a capture molecule to be bound to an IgG at the same time as an indicator molecule. The use of a reagent as a capture molecule or an indicator molecule depends upon whether the molecule is immobilized to a substrate when the molecule is exposed to an IgG. For example, a heavy chain of canine IgG molecule of the present invention is used as a capture molecule when the heavy chain of canine IgG molecule is bound on a substrate. Alternatively, a the heavy chain of canine IgG molecule is used as an indicator molecule when the heavy chain of canine IgG molecule is not bound on a substrate. Suitable molecules for use as capture molecules or indicator molecules include, but are not limited to, a the heavy chain of canine IgG molecule of the present invention, an antigen reagent or an anti-IgG antibody reagent of the present invention.

An immunoabsorbent assay of the present invention can further comprise one or more layers and/or types of secondary molecules or other binding molecules capable of detecting the presence of an indicator molecule. For example, an untagged (e.g., not conjugated to a detectable marker) secondary antibody that selectively binds to an indicator molecule can be bound to a tagged (e.g., conjugated to a detectable marker) tertiary antibody that selectively binds to the secondary antibody. Suitable secondary antibodies, tertiary antibodies and other secondary or tertiary molecules can be selected by those of skill in the art. Preferred secondary molecules of the present invention include an antigen, an anti-IgG idiotypic antibody and an anti-IgG isotypic antibody. Preferred tertiary molecules can be selected by a skilled artisan based upon the characteristics of the secondary molecule. The same strategy is applied for subsequent layers.

In one embodiment, the heavy chain of canine IgG molecule is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish well or a dipstick. A biological sample collected from an animal is applied to the substrate and incubated under conditions suitable to allow for the heavy chain of canine IgG molecule:test compound complex formation bound to the substrate. Excess non-bound

material, if any, is removed from the substrate under conditions that retain the heavy chain of canine IgG molecule:test compound complex binding to the substrate. An indicator molecule that can selectively bind to a test compound bound to the heavy chain of canine IgG molecule is added to the substrate and incubated to allow formation of a complex between the indicator molecule and the heavy chain of canine IgG molecule:test compound complex. Preferably, the indicator molecule is conjugated to a detectable marker (preferably to an enzyme label, to a colorimetric label, to a fluorescent label, to a radioisotope, or to a ligand such as of the biotin or avidin family). Excess indicator molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for analysis. Preferred test compounds to detect are antigens or anti-IgG antibodies.

In one embodiment, an immunosorbent assay of the present invention does not utilize a capture molecule. In this embodiment, a test sample is applied to a substrate, such as a microtiter dish well or a dipstick, and incubated under conditions suitable to allow for the test compound binding to the substrate. Any test compound is immobilized on the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain test compound binding to the substrate. A the heavy chain of canine IgG molecule is added to the substrate and incubated to allow formation of a complex between the heavy chain of canine IgG molecule and the test compound. Preferably, the heavy chain of canine IgG molecule is conjugated to a detectable marker (preferably to biotin, an enzyme label or a fluorescent label). Excess the heavy chain of canine IgG molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for analysis. Preferred test compounds to detect are antigens or anti-IgG antibodies.

Another preferred method to detect a test compound is a lateral flow assay, examples of which are disclosed in U.S. Patent No. 5,424,193, issued June 13, 1995, by Pronovost et al.; U.S. Patent No. 5,415,994, issued May 16, 1995, by Imrich et al; WO 94/29696, published December 22, 1994, by Miller et al.; and WO 94/01775, published January 20, 1994, by Pawlak et al.; each of these patent publications is incorporated by reference herein in its entirety. In one embodiment, a biological sample is placed in a lateral flow apparatus that includes the following components: (a) a support structure

defining a flow path; (b) a labeling reagent comprising a heavy chain of canine IgG, the labeling reagent being impregnated within the support structure in a labeling zone; and (c) a capture reagent comprising an anti- heavy chain of canine IgG antibody. The capture reagent is located downstream of the labeling reagent within a capture zone fluidly connected to the labeling zone in such a manner that the labeling reagent can flow from the labeling zone into the capture zone. The support structure comprises a material that does not impede the flow of the beads from the labeling zone to the capture zone. Suitable materials for use as a support structure include ionic (e.g., anionic or cationic) material. Examples of such a material include, but are not limited to, nitrocellulose (NC), PVDF, carboxymethylcellulose (CM). The support structure defines a flow path that is lateral and is divided into zones, namely a labeling zone and a capture zone. The apparatus can further comprise a sample receiving zone located along the flow path, more preferably upstream of the labeling reagent. The flow path in the support structure is created by contacting a portion of the support structure downstream of the capture zone, preferably at the end of the flow path, to an absorbent capable of absorbing excess liquid from the labeling and capture zones.

In this embodiment, the biological sample is applied to the sample receiving zone which includes a portion of the support structure. The labeling zone receives the sample from the sample receiving zone which is directed downstream by the flow path. The labeling zone comprises the heavy chain of canine IgG. A preferred labeling reagent is the heavy chain of canine IgG conjugated, either directly or through a linker, to a plastic bead substrate, such as to a latex bead. The substrate also includes a detectable marker, preferably a colorimetric marker. Typically, the labeling reagent is impregnated to the support structure by drying or lyophilization. The sample structure also comprises a capture zone downstream of the labeling zone. The capture zone receives labeling reagent from the labeling zone which is directed downstream by the flow path. The capture zone contains the capture reagent, in this case an anti-the heavy chain of canine IgG antibody, as disclosed above, that immobilizes the IgG complexed to the anti-IgG in the capture zone. The capture reagent is preferably fixed to the support structure by drying or lyophilizing. The labeling reagent accumulates in the capture zone and the accumulation is assessed visually or by an optical detection device.

Yet another embodiment of the present invention is a therapeutic composition that, when administered to a canid in an effective manner, is capable of protecting that animal from a disease mediated by canine IgE, such as, for example, allergy or inflammation. Therapeutic compositions of the present invention include therapeutic (protective or regulatory) compounds that are capable of regulating IL-13 amounts and/or activity. A therapeutic compound of the present invention is capable of regulating IL-13 activity and availability. Examples of regulatory compounds related to IL-13Rα proteins of the present invention include an isolated antibody that selectively binds to a canine IL-13Ra protein or other inhibitors or activators of IL-13Rα protein activity or amount. As such, these regulatory compounds may include antibodies, peptides, substrate analogs, and other large or small molecules which can be organic or inorganic. As used herein, a protective compound refers to a compound, that when administered to an animal in an effective manner, is able to treat, ameliorate, and/or prevent a disease mediated by IgE.

The efficacy of a therapeutic composition of the present invention to protect an animal from a disease mediated by IL-13 can be tested in a variety of ways including, but not limited to, detection of protective antibodies (using, for example, proteins or mimetopes of the present invention), detection of the amount of IL-13, or detection of cellular immunity within the treated animal. In one embodiment, therapeutic compositions can be tested in animal models such as mice. Such techniques are known to those skilled in the art.

One embodiment of the present invention is a therapeutic composition that, when administered to a canid, reduces allergy or inflammation present in said canid, said therapeutic composition comprising an excipient and a therapeutic compound selected from the group consisting of:

(a) an isolated protein selected from the group consisting of:(i) a protein comprising an at least 40 contiguous amino acid region identical in sequence to an at least 40 contiguous amino acid region selected from the group consisting of SEQ ID NO:50, SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:61, SEQ ID NO:66, and/or SEQ ID NO:69; and (ii) a protein comprising an amino acid sequence that is at least 70 percent identical a sequence selected from the group consisting of SEQ ID NO:50, SEQ ID NO:55, SEQ ID NO:58,

SEQ ID NO:61, SEQ ID NO:66, and/or SEQ ID NO:69; (iii) a fusion protein comprising:(a) a carrier protein; and ((b)) a protein selected from the group consisting of: ((i)) a protein comprising an at least 30 contiguous amino acid region identical in sequence to an at least 30 contiguous amino acid region selected from the group consisting of SEQ ID NO:50, SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:61, SEQ ID NO:66, and/or SEQ ID NO:69; and ((ii)) a protein comprising an amino acid sequence that is at least 80 percent identical a sequence selected from the group consisting of SEQ ID NO:50, SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:61, SEQ ID NO:66, and/or SEQ ID NO:69; and (iv) any other IL-13Rα protein (including a fusion protein) of the present invention;

(b) a mimetope of any of the proteins;

(c) a multimeric form of any of the proteins; (d) an isolated nucleic acid molecule comprising an IL-13Rα nucleic acid molecule, including an IL-13Rα2 chimeric nucleic acid molecule of the present invention;(e) an antibody that selectively binds any of the proteins of the present invention; and (f) an inhibitor identified by its ability to inhibit the activity of any of the proteins of the present invention.

The present invention also includes a therapeutic composition comprising at least one therapeutic compound of the present invention in combination with at least one additional compound protective against allergy or inflammation. Examples of such protective compounds include anti-inflammatory steroids, antihistamines, and anti-IgE antibodies.

In one embodiment, a therapeutic composition of the present invention can be used to protect an animal from a disease mediated by IL-13 by administering a IL-13Rα therapeutic composition to a canid in order to prevent undesirable IgE levels. Such administration can include, but is not limited to, oral, intravenous, intramuscular, intra ocular, mucosal, intranasal, subcutaneous, or transdermal application. A preferred route of administration is subcutaneous. In order to protect an animal from a disease mediated by IgE, a therapeutic composition of the present invention is administered to the animal in an effective manner such that the composition is capable of protecting that animal from

a disease mediated by IgE. Therapeutic compositions of the present invention can be administered to animals prior to disease in order to prevent disease and/or can be administered to animals after disease occurs. The exact dose, administration regimen, and administration route of therapeutic compositions of the present invention can be determined by one skilled in the art.

Therapeutic compositions of the present invention can be formulated in an excipient that the animal to be treated can tolerate. Examples of such excipients include water, saline, Ringer's solution, dextrose solution, Hank's solution, and other aqueous physiologically balanced salt solutions. Nonaqueous vehicles, such as fixed oils, sesame oil, ethyl oleate, or triglycerides may also be used. Other useful formulations include suspensions containing viscosity enhancing agents, such as sodium carboxymethylcellulose, sorbitol, or dextran. Excipients can also contain minor amounts of additives, such as substances that enhance isotonicity and chemical stability. Examples of buffers include phosphate buffer, bicarbonate buffer and Tris buffer, while examples of preservatives include thimerosal, or o-cresol, formalin and benzyl alcohol. Standard formulations can either be liquid injectables or solids which can be taken up in a suitable liquid as a suspension or solution for injection. Thus, in a non-liquid formulation, the excipient can comprise dextrose, human serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

In one embodiment of the present invention, a therapeutic composition can include an adjuvant. Adjuvants are agents that are capable of enhancing the immune response of an animal to a specific antigen. Suitable adjuvants include, but are not limited to, cytokines, chemokines, and compounds that induce the production of cytokines and chemokines (e.g., granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), colony stimulating factor (CSF), erythropoietin (EPO), interleukin 2 (IL-2), interleukin-3 (IL-3), interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 6 (IL-6), interleukin 7 (IL-7), interleukin 8 (IL-8), interleukin 10 (IL-10), interleukin 12 (IL-12), interferon gamma, interferon gamma inducing factor I (IGIF), transforming growth factor beta, RANTES (regulated upon activation, normal T cell expressed and presumably secreted), macrophage inflammatory proteins (e.g., MIP-1 alpha and MIP-1 beta), and

Leishmania elongation initiating factor (LEIF); bacterial components (e.g., endotoxins, in particular superantigens, exotoxins and cell wall components); aluminum-based salts; calcium-based salts; silica; polynucleotides; toxoids; serum proteins, viral coat proteins; block copolymer adjuvants (e.g., Hunter's Titermax™ adjuvant (Vaxcel™, Inc. Norcross, GA), Ribi adjuvants (Ribi ImmunoChem Research, Inc., Hamilton, MT); and saponins and their derivatives (e.g., Quil A (Superfos Biosector A/S, Denmark). Protein adjuvants of the present invention can be delivered in the form of the protein themselves or of nucleic acid molecules encoding such proteins using the methods described herein.

In one embodiment of the present invention, a therapeutic composition can include a carrier. Carriers include compounds that increase the half-life of a therapeutic composition in the treated animal. Suitable carriers include, but are not limited to, polymeric controlled release vehicles, biodegradable implants, liposomes, bacteria, viruses, other cells, oils, esters, and glycols.

One embodiment of the present invention is a controlled release formulation that is capable of slowly releasing a composition of the present invention into an animal. As used herein, a controlled release formulation comprises a composition of the present invention in a controlled release vehicle. Suitable controlled release vehicles include, but are not limited to, biocompatible polymers, other polymeric matrices, capsules, microcapsules, microparticles, bolus preparations, osmotic pumps, diffusion devices, liposomes, lipospheres, and transdermal delivery systems. Other controlled release formulations of the present invention include liquids that, upon administration to an animal, form a solid or a gel *in situ*. Preferred controlled release formulations are biodegradable, i.e., bioerodible.

Naked nucleic acid molecules of the present invention can be administered by a variety of methods. Suitable delivery methods include, for example, intramuscular injection, subcutaneous injection, intradermal injection, intradermal scarification, particle bombardment, oral application, and nasal application, with intramuscular injection, intradermal injection, intradermal scarification and particle bombardment being preferred. A preferred single dose of a naked DNA molecule ranges from about 1 nanogram (ng) to about 1 milligram (mg), depending on the route of administration and/or method of delivery, as can be determined by those skilled in the art. Examples of

administration methods are disclosed, for example, in U.S. Patent No. 5,204,253, by Bruner, et al., issued April 20, 1993, PCT Publication No. W0 95/19799, published July 27, 1995, by McCabe, and PCT Publication No. WO 95/05853, published March 2, 1995, by Carson, et al. Naked DNA molecules of the present invention can be contained in an aqueous excipient (e.g., phosphate buffered saline) and/or with a carrier (e.g., lipid-based vehicles), or it can be bound to microparticles (e.g., gold particles).

According to one embodiment, a nucleic acid molecule of the present invention can be administered to an animal in a fashion to enable expression of that nucleic acid molecule into a protective protein or protective RNA, e.g., antisense RNA, ribozyme, triple helix forms or RNA drug, in the animal. Nucleic acid molecules can be delivered to an animal in a variety of methods including, but not limited to, (a) administering a naked, i.e., not packaged in a viral coat or cellular membrane, nucleic acid as a genetic vaccine, e.g., as naked DNA or RNA molecules, such as is taught, for example in Wolff et al., 1990, *Science 247*, 1465-1468, or (b) administering a nucleic acid molecule packaged as a recombinant virus vaccine or as a recombinant cell vaccine, i.e., the nucleic acid molecule is delivered by a viral or cellular vehicle.

Administration of soluble receptor protein of the present invention, more preferably the extracellular portion of the receptor, to an animal will result in a decrease of circulating IL-13, the IL-13 receptor acts as a sponge to remove IL-13 from the circulation.

A genetic, i.e., naked nucleic acid, vaccine of the present invention includes a nucleic acid molecule of the present invention and preferably includes a recombinant molecule of the present invention. A genetic vaccine of the present invention can comprise one or more nucleic acid molecules of the present invention operatively linked to a transcriptional control sequence. In one embodiment, genetic vaccines include at least a portion of a viral genome, i.e., a viral vector. Any suitable transcription control sequence can be used, including those disclosed as suitable for protein production. Particularly preferred transcription control sequences include cytomegalovirus immediate early, preferably in conjunction with Intron-A, Rous sarcoma virus long terminal repeat, and tissue-specific transcription control sequences, as well as transcription control sequences endogenous to viral vectors if viral vectors are used. The incorporation of a

"strong" polyadenylation signal is also preferred. Preferred viral vectors include those based on alphaviruses, poxviruses, adenoviruses, herpesviruses, picornaviruses, and retroviruses.

Genetic vaccines of the present invention can be administered in a variety of ways, with intramuscular, subcutaneous, intradermal, transdermal, intranasal and oral routes of administration being preferred. A preferred single dose of a genetic vaccine ranges from about 1 nanogram (ng) to about 600 μg, depending on the route of administration and/or method of delivery, as can be determined by those skilled in the art. Suitable delivery methods include, for example, by injection, as drops, aerosolized and/or topically. Genetic vaccines of the present invention can be contained in an aqueous excipient, e.g., phosphate buffered saline, alone or in a carrier, e.g., lipid-based vehicles.

A recombinant virus vaccine of the present invention includes a recombinant molecule of the present invention that is packaged in a viral coat and that can be expressed in an animal after administration. Preferably, the recombinant molecule is packaging- or replication-deficient and/or encodes an attenuated virus. A number of recombinant viruses can be used, including, but not limited to, those based on alphaviruses, poxviruses, adenoviruses, herpesviruses, picornaviruses, and retroviruses. Preferred recombinant virus vaccines are those based on alphaviruses, such as Sindbis virus, raccoon poxviruses, species-specific herpesviruses and species-specific poxviruses. An example of methods to produce and use alphavirus recombinant virus vaccines are disclosed in U.S. Patent No. 5,766,602, Xiong et al., issued June 16, 1998; U.S. Patent 5,753,235, Haanes et al., issued May 19, 1998; and U.S. Patent 5,804,197, Haanes et al., issued September 8, 1998, all of which are incorporated by reference herein in their entirety.

When administered to an animal, a recombinant virus vaccine of the present invention infects cells within the immunized animal and directs the production of a protective protein or RNA nucleic acid molecule that is capable of protecting the animal from a disease. For example, a recombinant virus vaccine comprising a canine IL-13R□ nucleic acid molecule of the present invention is administered according to a protocol that results in the animal producing a sufficient immune response to protect itself from a disease mediated by IL-13. A preferred single dose of a recombinant virus vaccine of the

present invention is from about 1 x 10^4 to about 1 x 10^8 virus plaque forming units (pfu) per kilogram body weight of the animal. Administration protocols are similar to those described herein for protein-based vaccines, with subcutaneous, intramuscular, intranasal and oral administration routes being preferred.

A recombinant cell vaccine of the present invention includes recombinant cells of the present invention that express at least one protein of the present invention. Preferred recombinant cells for this embodiment include *Salmonella*, *E. coli*, *Listeria*, *Mycobacterium*, *S. frugiperda*, yeast, (including *Saccharomyces cerevisiae and Pichia pastoris*), BHK, CV-1, myoblast G8, COS, e.g.,COS-7, Vero, MDCK and CRFK recombinant cells. Recombinant cell vaccines of the present invention can be administered in a variety of ways but have the advantage that they can be administered orally, preferably at doses ranging from about 10^8 to about 10^{12} cells per kilogram body weight. Administration protocols are similar to those described herein for protein-based vaccines. Recombinant cell vaccines can comprise whole cells, cells stripped of cell walls or cell lysates.

A IL-13Rα inhibitor of the present invention is identified by its ability to bind to, modify, or otherwise interact with, a IL-13Rα protein, thereby inhibiting the activity of IL-13Rα. Suitable inhibitors of IL-13Rα activity are compounds that inhibit IL-13Rα protein activity in at least one of a variety of ways: (1) by binding to or otherwise interacting with or otherwise modifying the IL-13Rα binding, i.e. ligand binding, site, (2) by interacting with other regions of the IL-13Rα protein to inhibit IL-13Rα activity, for example, by allosteric interaction, and (3) by binding to or otherwise interacting with or otherwise modifying a IL-13Rα receptor binding site such that IL-13 is less likely to bind to the IL-13Rα receptor binding site. Inhibitors of IL-13Rα are preferably relatively small compounds.

Canine IL-13Rα proteins of the present invention can be used to develop regulatory compounds including inhibitors and activators that, when administered to a canid in an effective manner, are capable of protecting that canid from disease mediated by IL-13Rα or IL-13. Preferred regulatory compounds derived from the present invention include inhibitors and activators. In accordance with the present invention, the ability of a regulatory compound, including an inhibitor or activator, of the present

invention to protect a canid from disease mediated by IL-13Rα or IL-13 refers to the ability of that protein to, for example, treat, ameliorate or prevent a disease mediated by IL-13Rα in that canid.

In one embodiment of the present invention a compound that inhibits the activity of a IL-13Rα protein is identified by a) contacting an isolated canine IL-13Rα protein with a putative inhibitory compound under conditions in which, in the absence of a compound, IL-13Rα protein has IL-13 binding activity; and (b) determining if a inhibitory compound inhibits IL-13 binding activity. Preferably such a method is also conducted in the presence of IL-13.

A variety of methods are known to one skilled in the art to detect binding of IL-13 to an IL-13Rα protein. Such methods include, but are not limited to an assay in which IL-13 and a IL-13Rα binding partner can interact and/or bind to each other, using, for example, the yeast two-hybrid system, see for example, Luban, et al. 1995, *Curr. Opin. Biotechnol.*, 6, 59-64; and identifying those proteins that specifically bind to the canine IL-13Rα protein binding domain. Additional methods to identify protein-protein interactions include Biacore® screening, confocal immunofluorescent microscopy, UV cross-linking, and immunoprecipitations. An example of a IL-13Rα protein binding domain is an IL-13Rα2-binding domain, and a protein that would bind to a IL-13Rα2-binding domain would be IL-13. Additional teachings of general characteristics of reagents for use in the detection of binding between two moieties (e.g., between IL-13 and its receptor) as well as methods to produce and use such reagents are disclosed, for example, in United States Patent No. 5,958,880, issued September 28, 1999, by Frank et al.; and PCT International Publication No. WO 99/54349, published October 28, 1999, by McCall et al.; each of these references is incorporated by reference herein in its entirety; furthermore, the disclosed reagents and methods are incorporated by reference herein in their entireties. It is to be noted that although the reagents and methods disclosed in each of the citations do not relate to the proteins, nucleic acid molecules, antibodies and inhibitors of the present invention per se, the disclosed reagents and methods are applicable by those skilled in the art to reagents, kits and detection methods of the present invention.

One embodiment of the present invention includes an assay kit to identify the

presence of an inhibitor of a canine IL-13Rα protein in a canid, comprising an isolated IL-13Rα protein, and a means for determining the inhibition of activity of IL-13Rα, wherein said means enables the detection of inhibition, wherein detection of inhibition identifies an inhibitor of the ability of canine IL-13Rα protein to bind IL-13. Such a kit preferably also includes IL-13, preferably canine IL-13.

The present invention also includes a method and kit to detect IL-13, preferably canine IL-13. Higher than normal levels of IL-13 indicate the presence of allergy or inflammation in a canid. Such methods and kits use a canine IL-13Rα protein, preferably a canine IL-13Rα2 protein, of the present invention and involve the formation and detection of a complex between any IL-13 in a sample and that IL-13Rα protein. General characteristics of methods and reagents to detect IL-13 are disclosed herein, e.g., in U.S. Patent No. 5,958,880, *ibid.*

Also provided are kits comprising a container comprising at least one composition selected from the group consisting of

(a) a nucleic acid molecule of the present invention,

(b) a protein encoded by a nucleic acid of the present invention,

(c) a chimera of the present invention

(d) a fusion protein of the present invention

(e) a fusion sequence of the present invention.

In broad terms, a kit may contain canine IgG DNA or antibodies to the heavy chain of canine IgG. A kit may be used to detect DNA which hybridizes to canine IgG nucleic acid molecule of the present invention or amplified (PCR) using a nucleic acid molecule of the present invention, or to detect the presence of the heavy chain of canine IgG protein or peptide fragments in a sample. Such characterization is useful for a variety of purposes including but not limited to forensic analyses and epidemiological studies. Alternatively, a kit may contain DNA molecules, RNA molecules, recombinant protein and antibodies of the present invention for the purpose of screening and measuring levels of the heavy chain of canine IgG DNA, the heavy chain of canine IgG RNA or the heavy chain of canine IgG protein. The recombinant proteins, DNA molecules, RNA molecules and antibodies lend themselves to the formulation of kits suitable for the detection and typing of the heavy chain of canine IgG. All of these kits

would comprise a compartmentalized carrier suitable to hold in close confinement at least one container. The carrier may also further comprise reagents such as recombinant the heavy chain of canine IgG protein or anti-the heavy chain of canine IgG antibodies suitable for detecting the heavy chain of canine IgG. The carrier may also contain a means for detection such as labeled antigen or enzyme substrates or the like. A preferred kit of the present invention further comprises a detection means including one or more antigens disclosed herein, an antibody capable of selectively binding to an IgG disclosed herein and/or a compound capable of binding to a detectable marker conjugated to a the heavy chain of canine IgG protein (e.g., avidin, streptavidin and ImmunoPure® NeutrAvidin when the detectable marker is biotin). Such antigens preferably induce IgG antibody production in animals including canines, canines and/or equines.

In particular, kits useful in vaccination, therapy, diagnosis, detection of IgG, detection of specific IgG subclasses, identification of diseases by subclass, and monitoring of immune response are provided.

The following examples illustrate the present invention without, however, limiting it. It is to be noted that the Examples include a number of molecular biology, microbiology, immunology and biochemistry techniques considered to be known to those skilled in the art. Disclosure of such techniques can be found, for example, in Sambrook et al., *ibid.*, and related references.

Example 1: Cloning The heavy chain of canine IgG

Canine IgG probe preparation:

Degenerate primer C-IgG330-F (designated as SEQ ID NO: 40) was designed based on the conserved regions of IgGs from human, mouse, pig and bovine.

A ~750 bp DNA fragment was amplified with C-IgG330-F and M13 Forward primers from canine spleen cell cDNA library in a "touch-down" PCR reaction. The reaction condition was 94°C for 8 min, 3 cycles of 94 C for 30 Sec, 58 C for 45 Sec and 72 C for 1.2 min, then annealing temperature changed from 58 C to 56, 54, 52, 50, 48 and 46 C step-wise. The reaction was carried out for 3 cycles for each annealing temperature and 25 cycles at 44 C. The amplified DNA fragment was inserted into TA vector (Invitrogen). Plasmids that carry PCR amplified DNA were purified for sequencing. Blast search of the

sequencing data indicated that the DNA fragment was coding for canine IgG.

cIg-13. DNA (designated as SEQ ID NO: 28; the reverse and complement of this sequence is SEQ ID NO: 30).The cDNA fragment was found to contain DNA sequence (578 bp) encoding for CH3 and part of CH2 domains of canine IgG, and 3' UTR

cIg-13.AMI (designated SEQ ID NO:29) is the decoded amino acid sequence (AA 141) for cIg-13.DNA;

Screening canine IgG from a canine spleen cell cDNA library:

PCR fragments encoding canine IgG were used as probes in southern blots for screening canine IgG clones from a canine spleen cell cDNA library. Two distinct IgG heavy chains and a light chain DNA sequences were identified in the screening. One of the two heavy chains was a full length canine IgG (clone 3523), and the other a partial canine IgG DNA sequence (4325-P) that encodes CH3, CH2, CH1 and part of variable region of the IgG.

3523.DNA (1654 bp; designated SEQ ID NO:4; the reverse and complement of SEQ ID NO:4 is SEQ ID NO: 6)

3523.AMI (AA: 468; designated SEQ ID NO: 5) is the coding region of 3523.DNA.

Table 2.

Clone 3523	VH	C_H1	Hinge	C_H2	C_H3
DNA (bp)	70 - 480	481 - 774	775 - 825	826 - 1143	1144 - 1473
Amino acid	1 - 137	138 - 235	236 - 252	253 - 358	359 - 468

Estimated molecular mass of canine IgG-3523 is about 51.7 kDa with pI about 6.55.

4325-P.DNA (1364 bp) is designated SEQ ID NO: 31; the reverse and complement of SEQ ID NO: 31 is SEQ ID NO: 33.

4325-P.AMI (AA: 392; designated SEQ ID NO: 32) is the coding region of 4325-P.DNA)

Table 3.

Clone 4325-P	V_H	C_H1	Hinge	C_H2	C_H3
DNA (bp)	1 - 179	180 - 473	474 - 539	540 - 857	858 - 1187
Amino acid	1-57	58-155	156-177	178-283	284-392

Light chain DNA (Lambda chain, 938 bp) is designated SEQ ID NO: 25 (the reverse and complement of SEQ ID NO: 25 is SEQ ID NO: 27).

Light chain (AA: 235; designated SEQ ID NO: 26) represents the coding region of Lambda chain from 49 to 756 bp). Estimated molecular mass of canine IgG Lambda light chain is about 24.7 kDa with pI about 4.97.

Specific canine IgG primers in the conserved regions of IgG heavy chains were designed based on canine IgG sequences:

-IgG-FWD1: 5'GCCCTCCAGCAGGTGGCCCAGCGAGACC3'; (SEQ ID NO: 41)

-IgG-REV1: 5'GGGGATGGCGGCAGGACATACAC3'; (SEQ ID NO: 42)

-IgG-REV2: 5'TTTACCCGGAGAATGGGAGAGGG3'; (SEQ ID NO: 43)

-IgG-REV3: 5'GGTCTGCGTGGGCCACCTGCTGGAGGGC3'; (SEQ ID NO: 44)

-IgG-REV4: 5'GGGTGGGGGGCTTGCTGGGTGCCGGGCG3'. (SEQ ID NO: 45)

The primers were used in PCRs for amplification of other canine IgG heavy chain subclasses.

PCR amplification of IgGs from canine B-cell and T-cell lymphoma samples: First strand cDNAs prepared from 18 different canine B cell lymphoma samples were used as the templates in PCR reactions with IgG-FWD1 and IgG-REV2 primer set. The reaction condition is 94 C for 5 min; then 32 cycles on 94 C for 45 Sec, 54 C for 45 Sec and 72 C for 45 Sec. The amplified DNA fragments from the PCR will contain hinge region, CH2 and CH3 domains of canine IgG heavy chain. In addition to the DNA sequences of IgGs identified from the canine spleen cell cDNA library, two new IgG with different DNA sequences on hinge region were identified from the B cell lymphoma samples.

DNA and encoded amino acid sequences of hinge region of canine IgG heavy chains:

The DNA sequence of 3523-hinge region is designated SEQ ID NO: 1; the

reverse complement of SEQ ID NO:1 is SEQ ID NO:3.

The amino acid sequence of 3523-hinge region is designated SEQ ID NO:2

The DNA sequence of 4325-hinge region is designated SEQ ID NO: 22; the reverse complement of SEQ ID NO:22 is SEQ ID NO:24.

The amino acid sequence of 4325-hinge region is designted SEQ ID NO: 23.

The DNA sequence of Bly8-hinge region is designated SEQ ID NO: 7; the reverse complement of SEQ ID NO: 7 is SEQ ID NO:9.

The amino acid sequence of Bly8-hinge region is designated SEQ ID NO:8.

The DNA sequence of Bly9-hinge region is designated SEQ ID NO: 13; the reverse complement of SEQ ID NO:13 is SEQ ID NO: 15.

The amino acid sequence of Bly9-hinge region is designated SEQ ID NO:14.

PCR reactions were carried out using 5' end of canine IgG forward primer K9IgG5' (designated SEQ ID NO: 46) and canine IgG reverse primer IgG-REV4 and IgG-REV2, respectively. The reaction condition was carried out for 1 cycle of 94 C for 5 min, 3 cycles of 94 C for 35 Sec, 58/56/54/52 C for 45 Sec and 72 C for 1.5 min, then 22 cycles of 94 C for 35 Sec, 50 C for 45 Sec and 72 C for 1.5 min.

4325.DNA (1453 bp; designated SEQ ID NO: 19) is a cDNA fragment of the PCR using K9IgG5' and IgG-REV4 primers. (The reverse complement of SEQ ID NO:19 is SEQ ID NO: 21)

4325.AMI (AA: 473; designated SEQ ID NO: 20) is the deduced amino acid sequence encoded by SEQ ID NO;19.

Table 4.

Clone 4325	V_H	C_H1	Hinge	C_H2	C_H3
DNA (bp)	32 - 445	446 - 739	740 - 805	806 - 1123	1124 - 1450
Amino acid	1 - 138	139 - 236	237 - 258	259 - 364	365 - 473

Estimated molecular mass of canine IgG-4325 is about 52 kDa with pI about 8.17.

Clone Bly-8 was the product of two PCR fragments, Bly8-5C and Bly8-3C. Bly8-5C was a PCR fragment amplified from B-cell lymphoma sample using K9IgG5 and IgG-REV2 primers.

Bly8-5C.DNA (1168 bp) is designated as SEQ ID NO: 34; the reverse complement of SEQ ID NO:34 is SEQ ID NO: 36.

Bly8-5C.AMI (AA: 373; designated as SEQ ID NO:35) is the deduced amino acid sequence encoded by SEQ ID NO:34.

Based on DNA sequence of Bly8-5C variable region, a specific primer , Bly822F, (designated SEQ ID NO: 47) was designed.

Bly8-3C was amplified by PCR from the same B-cell lymphoma sample using Bly822F and IgG-REV2 primers.

Bly-8-3C.DNA (1059 bp) is designated as SEQ ID NO: 37; the reverse complement of SEQ ID NO: 37 is SEQ ID NO:39.

Bly8-3C.AMI (AA: 350; designated SEQ ID NO: 38) is the ddeduced amino acid sequence encoded by the SEQ ID NO:37.

Overlapping of the identical region of DNA fragments of Bly8-5C and Bly8-3C, a consensus DNA sequence was generated (Bly8).

Bly8.DNA (1460 bp) is designated as SEQ ID NO: 10; the reverse complement of SEQ ID NO:10 is SEQ ID NO:12.

Bly8.AMI (AA: 470; designated as SEQ ID NO: 11) is the deduced amino acid sequence encoded by SEQ ID NO:10.

Table 5.

Clone Bly8	VH	C_H1	Hinge	C_H2	C_H3
DNA (bp)	48 - 464	465 - 758	759 - 809	810 - 1127	1128 - 1457
Amino acid	1 - 139	140 - 237	238 - 254	255 - 360	361 - 470

Estimated molecular mass of canine IgG-Bly8 is about 51.2 kDa with pI about 6.24.

Bly9.DNA (1456 bp; designated SEQ ID NO: 16) is a cDNA fragment of the PCR using K9IgG5' and IgG-REV4 primers); the reverse complement of SEQ ID NO:16 is SEQ ID NO:18.

Bly9.AMI (AA: 474; designated SEQ ID NO:17) is the deduced amino acid sequence encoded by SEQ ID NO:16.

Table 6.

Clone Bly9	VH	C_H1	Hinge	C_H2	C_H3
DNA (bp)	32 - 454	455 - 748	749 - 808	809 - 1126	1127 - 1453
Amino acid	1 - 141	142 - 239	240 - 259	260 - 365	366 - 474

Estimated molecular mass of canine IgG-Bly9 is about 51.8 kDa with pI about 6.15.

Example 2

This example describes the isolation and sequencing of nucleic acid molecules encoding canine IL-13 receptor α1 (i.e. nCaIL-13Rα1) nucleic acid molecules of the present invention.

A cDNA library was prepared from a canine PBMC cDNA library. The library was a *C. familiaris* mitogen activated PBMC cDNA library that was constructed in the Uni-Zap® XR Vector (available from Stratagene Cloning Systems, La Jolla, CA) using Stratagene's ZAP-cDNA® Synthesis Kit and the manufacturer's protocol. Two degenerate synthetic oligonucleotide primers were designed from the conserved regions of bovine, mouse and human IL-13 receptors (IL-13R): Primer 13R1F1, a sense primer corresponding to amino acid residues from 48 through 59 of human IL-13 receptor α1 denoted herein as SEQ ID NO:50 as found in U.S. Patent No. 5,710,023, ibid has the sequence 5' ATHTGGACNTGGAAYCCNCCNGARGGNGC 3', denoted herein as SEQ ID NO:36; Primer 13R1R1, a anti-sense primer corresponding to amino acid residues from 202 through 213 of the same human IL-13 receptor α1 has the sequence 5' ATYTTNCCNGCRTTRTCYTTNACCATDATYTGNAC 3', denoted herein as SEQ ID NO:84, where D represents A, T OR G, H represents A or C or T, N represents A or C or G or T, R represents A or G and Y represents C or T. PCR amplification of fragments from the PBMC cDNA library was conducted using touch-down PCR amplification conditions, which consist of 1 cycle at 94 °C for 8 min; 3 cycles of 94 °C for 30 seconds, 58 °C for 45 seconds and 72 °C for 1 min; then annealing temperature changed from 58 °C to 56 °C, 54 °C, 52 °C, 50 °C, 48 °C,46 °C and 44 °C. step-wise. The reaction was carried out for 3 cycles for each annealing temperature and 25 cycles at 44 °C. A PCR amplification product of about 500 base pairs (bp) was generated and is denoted herein as nCaIL-13Rα1$_{483}$.

The amplified DNA fragment was purified with Qiagen gel purification kit,

available from Qiagen, La Jolla, CA) and PCR products were cloned into the TA cloning vector (available from Invitrogen Corporation, Carlsbad, CA), and the resulting clones were sequenced using an ABI Prism™ Model 377 Automatic DNA Sequencer (available from Perkin-Elmer Applied Biosystems Inc., Foster City, CA). DNA sequencing reactions were performed using Prism™ dRhodamine Terminator Cycle Sequencing Ready Reaction kits (available from PE Applied Biosystems Inc.).

The sequencing results indicated that nCaIL-13Rα1$_{483}$ contained 483 nucleotides and coded for a portion of canine IL-13Rα1. The coding strand of nCaIL-13Rα1$_{483}$ was shown to have a nucleic acid sequence referred to herein as SEQ ID NO:48.

To identify a cDNA encoding a full-length canine IL-13 Rα1 protein, nCaIL-13Rα1$_{483}$ was labeled with ^{32}P and used as a probe to screen the canine PBMC cDNA library described above. Hybridization was done at about 6X SSC, 5X Denhardt's solution, 0.5 % SDS, 100 μg/ml of ssDNA at about 68°C, for about 36 hr. (the compositions of SSC, Denhardt's and ssDNA are described in Sambrook et al., ibid.). The filters were washed 3 times, for about 30 minutes per wash, at about 55° C in about 2X SSC, 0.2% SDS, followed by a final wash of about 30 minutes in the same buffer except using about 1X SSC. Ninety six positive clones were selected in the first screen. Sixty of 96 clones scored as positive by PCR when 13R1F1 and 13R1R1 primers were used in the reaction. Two positive clones (clone #44 and #48) from this screening were picked for excision using ExAssis™/SOLR™ system (available from Stratagene, La Jolla, CA); DNA was isolated from each clone, purified by mini-prep and submitted for sequencing. The cDNA insert of clone # 44 was sequenced for both strands using vector flanking primers and gene-specific internal primers. Sequence analysis was performed using the GAP program of GCG (available from the University of Wisconsin) using the alignment settings of: gap weight set at 50, length weight set at 3, and average match set at 10 for nucleic acid sequence comparisons; and gap weight set at 12, length weight set at 4, and average match set at 2.912 for amino acid sequence comparisons. The cDNA insert, determined to be 1547 bp in length, is referred to herein as nCaIL-13Rα1$_{1547}$, the coding strand of which was shown to have a nucleic acid sequence denoted herein as SEQ ID NO:49. The complement of SEQ ID NO:49 is represented herein by SEQ ID NO:51. Translation of SEQ ID NO:2 suggests that nucleic acid molecule nCaIL-

13Rα1$_{1547}$ encodes a full-length IL-13Rα1 protein of about 405 amino acids, denoted herein as PcaIL-13Rα1$_{405}$, the amino acid sequence of which is presented in SEQ ID NO:50, assuming an open reading frame spannning from nucleotide 1 to nucleotide 3 of SEQ ID NO:49 and a stop codon spanning from nucleotide 1216 through nucleotide 1218 of SEQ ID NO:49. The coding region encoding PcaIL-13Rα1$_{405}$ is presented herein as nCaIL-13Rα1$_{1215}$, which has the nucleotide sequence SEQ ID NO:52 (the coding strand) and SEQ ID NO:53 (the complementary strand).

Example 3

This example describes the isolation and sequencing of nucleic acid molecules encoding a canine IL-13 receptor α2 (i.e., nCaIL-13Rα2) nucleic acids molecules of the present invention.

The same PBMC cDNA library described in Example 1 and a canine mast cell cDNA library were used as templates for the amplification of nCaIL-13Rα2 nucleic acid molecules. The canine mast cell cDNA library was prepared as follows. Total RNA was extracted from approximately 7 X 10^8 freshly harvested canine mast cells using an acid-guanidinium-phenol-chloroform method similar to that described by Chomzynski et al., 1987, *Anal. Biochem 162*, 156-159. Poly A^+ selected RNA was separated from the total RNA preparation by oligo-dT cellulose chromatography using the mRNA Purification Kit (available from Pharmacia, Newark, NJ) according to the method recommended by the manufacturer. The canine mast cell cDNA library was constructed in lambda-Uni-ZAP® Synthesis Kit protocol (available from Stratagene, La Jolla, CA), using Stratagene's ZAP-cDNA Synthesis Kit protocol. Approximately 5 micrograms (μg) of mast cell Poly A^+ RNA was used to produce the mast cell cDNA library. Four degenerate synthetic oligonucleotide primers were designed from the conserved region of IL-13 receptor α2 chains from mouse and human: Primer 13R2F1D, a sense primer corresponding to amino acid residues from 28 through 40 of human IL-13 receptor chain denoted herein as SEQ ID NO:50 as found in U.S. Patent No. 5,710,023, ibid has the sequence 5'GARATHAARGTNAAYCCNCCNCARGAYTTYGARAT 3', denoted herein as SEQ ID NO:85. Primer 13R2F2D, a sense primer corresponding to amino acid residues from 91 through 100 of the same human IL-13 receptor α2 has the sequence 5'

TAYAARGAYGGNTTCTGAYYTNAAYAARGGNATHGA 3', denoted herein as SEQ ID NO:86. Primer 13R2R1D, a anti-sense primer corresponding to amino acid residues from 317 through 326 of the same human IL-13 receptor α2 has the sequence 5' CCAYTCNSWCCADATNCCRTCRTCNGCRCARTADATRTTNACYTT 3', denoted herein as SEQ ID NO:87, and primer 13R2R2D, another anti-sense primer corresponding to amino acid residues from 174 through 181 of the same human IL-13 receptor α2 has the sequence 5'GCRTGRTCNARNCCYTCRTACCA 3', also known as SEQ ID NO:88, where D represents A, T OR G, H represents A or C or T, N represents A or C or G or T, R represents A or G and Y represents C or T. PCR amplifications of DNA fragments from the cDNA libraries were conducted using the touch-down PCR amplification conditions as described in Example 1 with an M13 reverse primer (available from Stratagene Cloning Systems, La Jolla, CA) and the 13R2R1D primer. The reaction mix from the first PCR was used as the template in the second PCR with a T3 primer (available from Stratagene Cloning Systems, La Jolla, CA) and the 13R2R2D primer to amplify a DNA fragment of about 750 bp denoted herein as nCaIL-13Rα2_{620}.

The amplified DNA fragment was purified with the Qiagen gel purification kit, PCR products were cloned into the TA cloning vector and the resulting clones were sequenced as described in Example1.

The sequencing results indicated that nCaIL-13Rα2_{620} the cDNA contained 620 nucleotides and coded for the amino-terminal portion of a canine IL-13Rα2 protein. The coding strand of nCaIL-13Rα2_{620} was shown to have a nucleic acid sequence referred to herein as nCaIL-13Rα2_{620}, also denoted as SEQ ID NO:54. Translation of SEQ ID NO:54 shows that the nucleic acid nCaIL-13Rα2_{620} encodes a partial protein of 145 amino acids, denoted herein as PcaIL-13Rα2_{145}, the amino acid sequence of which is presented in SEQ ID NO:55, assuming an open reading frame having an initiation codon spanning from nucleotide 184 through nucleotide 186 of SEQ ID NO:54 and continuing through nucleotide 620 of SEQ ID NO:54. The complement of SEQ ID NO54 is represented herein by SEQ ID NO:56.

A similar PCR cloning approach was used to isolate a cDNA encoding the carbonyl terminal region of a canine IL-13Rα2 protein. The canine mast cell library was

used as the template. Two primer sets, 13R2F1D/T7 and 13R2F2D/KS, were used in the PCR. The reaction mix from the first PCR was used in the second PCR with 13R2F2D/13R2R1D and 13R2F2/13R2R1D primer sets, respectively. DNA fragments of predicted size were purified and submitted for sequencing. The sequencing result showed that the DNA fragments coded for canine IL13-Rα2 proteins.

Specific IL-13-Rα2 primers were designed based on the obtained canine IL-13-Rα2 DNA sequence. 13R2F5 with the DNA sequence of 5'AGCGGATCCCTCTATGCTTTCAAATGCTGAGATAAAAGTTAATCCTCCTCAG G 3', denoted herein as SEQ ID NO:89 and 13R2F2 with the sequence of 5'TGGACATCACCACAAGGAAATCGGG 3', denoted herein as SEQ ID NO:90. A PCR reaction mixture generated using 13R2F5/M13 forward primers was used as the template in a second PCR with 13R2F2 and T7 primers in a manner as described in Example 1. An approximately 900 bp DNA fragment was detected in the reaction. This DNA fragment, denoted herein as nCaIL-13Rα2_{878} was purified and subcloned into TA vector. The positive clones were purified and submitted for sequencing as described in Example1. The sequencing results indicated that the nCaIL-13Rα2_{878} molecule contained 878 nucleotides and coded for the carboxyl portion of canine IL-13Rα2 and also includes the untranslated terminal region (UTR). The coding strand of nCaIL-13Rα2_{878} was shown to have a nucleic acid sequence referred to herein as SEQ ID NO:57. Translation of SEQ ID NO:57 shows that the nucleic acid nCaIL-13Rα2_{878} encodes a protein of 255 amino acids, denoted herein as PCaIL-13Rα2_{255}, the amino acid sequence of which is presented in SEQ ID NO:58, assuming an open reading frame spanning from nucleotide 1 to nucleotide 3 of SEQ ID NO:57 and a stop codon spanning from nucleotide 766 through nucleotide 768 of SEQ ID NO:57 . The complement of SEQ ID NO:57 is represented herein by SEQ ID NO:59.

A cDNA encoding a full-length canine IL-13Rα2 protein was constructed by lining up nCaIL-13Rα2_{620} and nCaIL-13Rα2_{878} to form a consensus nucleic acid molecule sequence, referred to herein as nCaIL-13Rα2_{1454}, the coding strand of which has a nucleic acid sequence denoted herein as SEQ ID NO:60 and the complementary strand which has a nucleic acid sequence represented herein by SEQ ID NO:62.

Translation of SEQ ID NO:60 suggests that nucleic acid molecule nCaIL-13Rα2$_{1454}$ encodes a full-length IL-13Rα2 protein of 386 amino acids, denoted herein as PcaIL-13Rα2$_{386}$, the amino acid sequence of which is presented in SEQ ID NO:61, assuming an open reading frame having an initiation codon spanning from nucleotide 184 through nucleotide 186 of SEQ ID NO:60 and a stop codon spanning from nucleotide 1342 through nucleotide 1345 of SEQ ID NO:60. The coding region encoding PcaIL-13Rα2$_{386}$ is presented herein as nCaIL-13Rα2$_{1158}$, which has the nucleotide sequence SEQ ID NO:63 (the coding strand) and SEQ ID NO:64 (the complementary strand). A putative signal sequence coding region extends from nucleotide 184 through nucleotide 246 of SEQ ID NO:60. The proposed mature protein (i.e., canine IL-13Rα2 protein from which the signal sequence has been cleaved), denoted herein as PCAIL-13Rα2$_{365}$, contains about 365 amino acids, extending from residue 22 through residue 386 of SEQ ID NO:61; amino acid sequence of PCaIL-13Rα2$_{365}$ is represented herein as SEQ ID NO:66. The nucleic acid molecule encoding PCaIL-13Rα2$_{365}$ is denoted herein as nCaIL-13Rα2$_{1095}$, extending from nucleotide 247 through nucleotide 1345 of SEQ ID NO:60. nCaIL-13Rα2$_{1095}$ has a coding sequence denoted SEQ ID NO:65 and a complementary sequence denoted SEQ ID NO:67. PCaIL-13Rα2$_{386}$ has an apparent extracellular domain, extending from residue S-22 to T-338 of SEQ ID NO:61, denoted herein as PCaIL-13Rα2$_{318}$, represented herein by SEQ ID NO:69. The nucleic acid molecule encoding PCaIL-13Rα2$_{318}$ is denoted herein as nCaIL-13Rα2$_{954}$, extending from nucleotide 247 through nucleotide 1197 of SEQ ID NO:60. nCaIL-13Rα2$_{954}$ has a coding sequence denoted SEQ ID NO:68 and a complementary sequence represented herein by SEQ ID NO:70. PCaIL-13Rα2$_{318}$, represented herein by SEQ ID NO:69. The nucleic acid molecule nCaIL-13Rα2$_{954}$, herein denoted SEQ ID NO:69, when expressed in *Escherichia coli* is processed with an additional methionine (ATG) start sequence on the amino terminal end of the nucleic acid sequence. Translation of nCaIL-13Rα2$_{954}$ results in a protein PCaIL-13Rα2$_{318}$ which also contains the additional methionine at the amino terminal end.

Example 4

This example describes the preparation of canine IL-13Rα2-Fc chimeric nucleic

acid molecules, recombinant molecules and recombinant cells as well as expression and biological activity of respective fusion proteins of the present invention. A. Construction of canine IL-13Rα2 chimeric nucleic acid molecules, recombinant molecule sequences and recombinant cells.

In order to create a canine chimeric nCaIL13-Rα2-Fc nucleic acid molecules, four specific primers were designed based on nCaIL13-Rα2$_{954}$ and canine IgE-Fc nucleic acid sequences. Canine IgE-Fc nucleic acid and amino acid sequences are disclosed in U.S.C.A. entitled "CANINE IMMUNOGLOBULIN G MOLECULES AND RELATED METHODS", filed April 7, 2000 by Tang referred to The following primers were constructed: primer 13R2FcF (forward primer containing an NdeI site), with the sequence 5'GCACATATGTCTATGCTTTCAAATGCTGAATAAAAGTTAATCCTCCTCAGG3', denoted SEQ ID NO:91; primer 13R2FcR2 (reverse primer containing a BamHI site), with the sequence 5'AAAGGATCCGGTTTCCTTCCAGATATCATTTCCAGC3', represented herein as SEQ ID NO:92; primer CIgGFcF (forward primer containing a BamHI site), having the sequence 5'CCGGGATCCAACACTAAAGTAGACAAGCGTG 3', represented herein as SEQ ID NO:93; and primer cIgGFcR (reverse primer containing a XhoI site), having the sequence 5' GCGCTCGAGTCATTTACCCGGAGAATGGGAGGG 3', represented herein as SEQ ID NO:94.

A nCaIL13-Rα2$_{954}$ nucleic acid molecule (with coding strand of SEQ ID NO:68) that encodes an extracellular portion of canine IL-13Rα2 DNA (from S^{22} to T^{338}) was PCR amplified from the mast cell cDNA library described in Example 2 using the above primers to introduce restriction sites NdeI and BamHI, the resulting product was digested with NdeI and BamHI; restriction enzymes (available from New England Biolabs, Inc., Beverly, MA). NdeI / BamHI digested nucleic acid molecule nCaIL13-Rα2$_{954}$ was fractionated on a 1% agarose gel, purified with Qiagen gel purification kit (available from Qiagen, La Jolla, Ca.) and subcloned into similarly cleaved plasmid λPRcro/T2ori/RSET-B, produced as described in PCT Patent Publication No. WO; 98/12563, published March 26, 1998 by Grieve, et al. After confirmation of the correct ligation of NdeI / BamHI

digested canine nCaIL13-R$\alpha 2_{954}$ into the λPRcro/T2ori/RSET-B plasmid by DNA sequencing, λPRcro/T2ori/RSET-B vector containing nCaIL13-R$\alpha 2_{954}$ referred to herein as recombinant molecule pλp_R-nCaIL13-R$\alpha 2_{954}$ was digested with BamHI and XhoI restriction enzymes. Nucleic acid molecules encoding four canine IgG-Fc partial-length proteins were constructed by combining a nCa IL-13R$\alpha 2_{954}$ fragment of the BamHI/XhoI digest with a IgG-Fc fragment, produced by methods of which are described herein, as follows:

Nucleic acid molecule nCaIL-13Rα2-Fc-3523_{1683} includes nucleic acid molecule nCaIL-13R$\alpha 2_{954}$ linked to a canine Ig gamma chain nucleic acid molecule the coding strand of which includes nucleotides 748 through 1473 of SEQ ID NO:51. Fusion protein PCaIL-13Ra2-Fc-3523 includes PCaIL-13R$a2_{318}$ linked to a canine Ig gamma chain protein that includes amino acids 227 through 468 of SEQ ID NO:52.

Chimeric nCaIL-13Rα2-Fc-3523_{1683}, in which NdeI/BamHI digested nCaIL-13R$\alpha 2_{954}$ is ligated to nCaFcγ3523_{1473}, has a coding strand the nucleic acid sequence of which is represented by SEQ ID NO:71 and a complementary strand the nucleic acid sequence of which is represented by SEQ ID NO:73.

Translation of SEQ ID NO:71 shows that the nucleic acid molecule nCaIL-13Rα2-Fc-3523_{1683} encodes a fusion protein of 561 amino acids, denoted herein as PcaIL-13Rα2-Fc-3523_{561}.

The chimeric nucleic acid molecule was ligated into λPRcro/T2ori/RSET-B to form recombinant molecule nCaIL-13Rα2-Fc-3523_{1683}, which was transformed into *E. coli* to form recombinant cell nCaIL-13Rα2-Fc-3523_{1683}.

Nucleic acid molecule nCaIL-13Rα2-Fc-4325_{1695} includes nucleic acid molecule nCaIL-13R$\alpha 2_{954}$ linked to a canine Ig gamma chain nucleic acid molecule the coding strand of which includes nucleotides 713 through 1450 of SEQ ID NO:66.

Fusion protein PCaIL-13Rα2-Fc-4325 includes PCaIL-13R$\alpha 2_{318}$ linked to a canine Ig gamma chain protein that includes amino acids 228 through 473 of SEQ ID NO:67.

Chimeric nCaIL-13Rα2-Fc-4325_{1695}, in which NdeI/BamHI digested nCaIL-13R$\alpha 2_{954}$ is ligated to nCaFcγ4325_{1450}, has a coding strand the nucleic acid sequence of which is represented by SEQ ID NO:74 and a complementary strand the nucleic acid

sequence of which is represented by SEQ ID NO:76.

Translation of SEQ ID NO:74 shows that the nucleic acid molecule nCaIL-13Rα2-Fc-4325$_{1695}$ encodes a fusion protein of 565 amino acids, denoted herein as PCaIL-13Rα2-Fc-4325$_{565}$.

The chimeric nucleic acid molecule was ligated into λPRcro/T2ori/RSET-B to form recombinant molecule nCaIL-13Rα2-Fc-4325$_{1695}$, which was transformed into *E. coli* to form recombinant cell nCaIL-13Rα2-Fc-4325$_{1695}$.

Nucleic acid molecule nCaIL-13Rα2-Fc-B9$_{1689}$ includes nucleic acid molecule nCaIL-13Rα2$_{954}$ linked to a canine Ig gamma chain nucleic acid molecule the coding strand of which includes nucleotides 725 through 1453 of SEQ ID NO:63.

Fusion protein PCaIL-13Rα2-Fc-B9$_{563}$ includes PCaIL-13Rα2$_{318}$ linked to a canine Ig gamma chain protein that includes amino acids 232 through 474 of SEQ ID NO:64.

Chimeric nCaIL-13Rα2-Fc-B9$_{1689}$, in which NdeI/BamHI digested nCaIL-13Rα2$_{954}$ is ligated to nCaFcγ B9$_{474}$, has a coding strand the nucleic acid sequence of which is represented by SEQ ID NO:77 and a complementary strand the nucleic acid sequence of which is represented by SEQ ID NO:79.

Translation of SEQ ID NO:77 shows that the nucleic acid molecule nCaIL-13Rα2-Fc-B9$_{1689}$ encodes a fusion protein of 563 amino acids, denoted herein as PCaIL-13Rα2-Fc-B9$_{563}$.

The chimeric nucleic acid molecule was ligated into λPRcro/T2ori/RSET-B to form recombinant molecule nCaIL-13Rα2-Fc-B9$_{1689}$, which was transformed into *E. coli* to form recombinant cell nCaIL-13Rα2-Fc-B9$_{1689}$.

Nucleic acid molecule nCaIL-13Rα2-Fc-B8$_{1683}$ includes nucleic acid molecule nCaIL-13Rα2$_{954}$ linked to a canine Ig gamma chain nucleic acid molecule the coding strand of which includes nucleotides 732 through 1457 of SEQ ID NO:57.

Fusion protein PCaIL-13Rα2-Fc-B8$_{561}$ includes PCaIL-13Rα2$_{318}$ linked to a canine Ig gamma chain protein that includes amino acids 229 through 470 of SEQ ID NO:58.

Chimeric nCaIL-13Rα2-Fc-B8$_{1683}$, in which NdeI/BamHI digested nCaIL-13Rα2$_{954}$ is ligated to nCaFcγ B9$_{474}$, has a coding strand the nucleic acid sequence of

which is represented by SEQ ID NO:80 and a complementary strand the nucleic acid sequence of which is represented by SEQ ID NO:82.

Translation of SEQ ID NO:80 shows that the nucleic acid molecule nCaIL-13Rα2-Fc-$B8_{1683}$ encodes a fusion protein of 561 amino acids, denoted herein as PcaIL-13Rα2-Fc-$B8_{561}$.

The chimeric nucleic acid molecule was ligated into λPRcro/T2ori/RSET-B to form recombinant molecule nCaIL-13Rα2-Fc-$B8_{1683}$, which was transformed into *E. coli* to form recombinant cell nCaIL-13Rα2-Fc-$B8_{1683}$.

B Expression, refolding and biological activity of IL-13Rα2-Fc fusion proteins.

Recombinant cells nCaIL-13Rα2-Fc-3523_{1683}, nCaIL-13Rα2-Fc-4325_{1695}, nCaIL-13Rα2-Fc-$B9_{1689}$, and nCaIL-13Rα2-Fc-$B8_{1683}$ produced as described in Example 3A were each cultured at 30 °C for 5 hours until the culture reached an OD of expression of 1.2 the fusion protein was induced by changing the culture temperature to 42 °C for 3 hours. Cell pellets were then collected by centrifugation at 3000 x g. Each of the four recombinant cells expressed its respective fusion protein as determined by appearance of the correct molecular weight band on an SDS-polyacrylamide gel using standard conditions and protein markers.

Fusion protein PCaIL-13Rα2-Fc-$B9_{563}$ (SEQ ID NO:78) was solubilized by the following method: the cell pellet was resuspended in cracking buffer (25 mM Tris-HCl pH 7.5, available from Sigma, St. Louis, MO) at 100 mg/ml (milligrams per milliliter) and homogenized with a polytron (available from Brinkman Instrument, Westbury, NY). The cells were broken using a microfluidizer (available from Microfluidics, Newton, MA) at 120 psi by recirculating 20 ml batches of resuspended cells for 30 pulses. The broken cell suspension was centrifuged at 30,000 times gravity (xg) for 30 minutes. The cell pellet from this centrifugation was resuspended (same volume as above) in a buffer containing 25 mM Tris-HCl pH 7.5, 1% deoxycholate, and 1% Triton X-100 (all available from Sigma), and rocked for 30 minutes at 4°C, centrifuged at 30,000 xg for 30 minutes, the pellet was resuspended in cracking buffer (above), and recentrifuged at 1000 x g for 5 minutes. The supernatant from the low-speed centrifugation (1000 x g)was then recentrifuged at 30,000 x g for 30 minutes. The pellet was resuspended (same volume as above) in 25 mM Tris-HCl pH 9.5, 8 M urea, and 50 mM beta-mercaptoethanol (all

available from Sigma). The resuspended inclusion bodies were incubated at 4°C for 30 minutes, recentrifuged at 30,000 x g for 30 minutes, yielding a supernatant that contains solubilized IL-13Rα2-Fc fusion protein. The protein concentration of the supernatant containing the fusion protein as determined by absorbance at 280 nanometers (nm), was approximately 1 mg/ml.

Using solubilized chimera PCaIL-13Rα2-Fc-$B9_{563}$, a number of different refolding conditions were tried. To 10 mls of resolubilized PCaIL-13Rα2-Fc-$B9_{563}$, 1.1 ml of a buffer in Column A of Table 7 was added to the solubilized IL-13Rα2-Fc-$B9_{563}$ such that final concentrations as noted in Table 7 were achieved. The resulting mixture was incubated at 30°C for 2 hours, then 120 ml of a buffer from Column B of Table 7 was added, to give final concentrations as noted in Table 7. This mixture was incubated overnight at 4°C. The resulting material was then placed into 12,000 MWCO dialysis membranes (available from Spectrum, Gardena, CA), and dialyzed overnight at 4°C against phosphate buffered saline (PBS pH 7.5), with three changes of 4 liters each. The dialyzed protein was centrifuged at 10,000 x g for 30 minutes, and the supernatant was collected.

The ability of PCaIL13-Rα2-Fc fusion protein refolded under each set of conditions to inhibit the biological activity of recombinant canine IL-13 was determined by TF-1 cell proliferation. TF-1 cells (available from R&D Systems, Minneapolis, MN), a human erthyroleukemia cell line, were maintained in RPMI-1640 media (available from Sigma Chemical Corp) supplemented with 2 mM L-glutamine (available from Life Technologies, Gaithersburg, MD), 5 microgram (μg)/ml gentamycin (available from Sigma Chemical Corp), 5% fetal bovine serum (available from Summit, Fort Collins, CO), and 2 nanogram/ml recombinant human GM-CSF (rhuGM-CSF, available from R&D systems), referred to as Complete Culture Medium. Cells were cultured in a humidified incubator, maintained at 37°C with a mix of 5% CO_2 and 95% ambient air. PCaIL13-Rα2-Fc fusion protein supernatants, produced as described above and canine IL-13 (prepared as described in U.S. Serial No. 09/322,409, filed May 28, 1999) were each diluted to the appropriate concentration in Complete Culture Medium (see above), without rhuGM-CSF, and filter sterilized. The diluted IL-13 and fusion protein solutions were then mixed at an initial cytokine to receptor molar ratio of either 1:25 (6.6 ug/ml for

IL-13α2-Fc, and 50 ng/ml IL-13) or 1:125 cytokine to receptor (6.6 ug/ml for IL-13α2-Fc, and 10 ng/ml IL-13) and incubated overnight at 4°C in a sterile, 96-well flat bottom tissue culture plate (available from Beckton Dickson Labware, Franklin Lakes, NJ). Cultured TF-1 cells were then extensively washed to remove rhuGM-CSF and added at 1 X 10^4 cells per well. Cells were incubated with the recombinant canine IL-13/ IL-13Rα2-Fc fusion protein mixture as described above (except in the absence of rhuGM-CSF) for 48 hours, then pulsed with 1 microcurie per well tritiated thymidine (available from ICN Pharmaceuticals, Irvine, CA) and incubation continued for another 18 hours. Contents of the wells were harvested onto glass fiber filters (available from Wallac, Inc., Gaithersburg, MD), and counted in a Wallac Trilux 1450 scintillation counter (available from Wallac Inc).

Table 7. Refolding conditions and resultant activity for PCaIL-13Rα2-Fc-$B9_{563}$.

Condition	Buffer A	Buffer B	Percent inhibition of IL-13 stimulated TF-1 cell proliferation; IL-13 at 50 ng/ml	Percent inhibition of IL-13 stimulated TF-1 cell proliferation; IL-13 at 10 ng/ml
1A	25 mM GSH, 6 mM DTT 0.05% Tween 80, 1 mM EDTA	50 mM Tris pH 10, 12.5 mM cysteine, 2 mM EDTA	29.4	30.9
1B	25 mM GSH, 6 mM DTT 0.05% Tween 80, 1 mM EDTA	50 mM Tris pH 8, 12.5 mM cysteine, 2mM EDTA	34.6	43.7
1C	25 mM GSH, 6 mM DTT 0.05% Tween 80, 1 mM EDTA	50 mM Tris pH 10, 2.7 mM GSSG, 0.8 mM EDTA	92.3	100
1D	25 mM GSH, 6 mM DTT 0.05% Tween 80, 1 mM EDTA	50 mM Tris pH 8, 2.7 mM GSSG, 0.8 mM EDTA	31.7	99.8
2A	25 mM GSSG, 6 mM DTT, 0.05% Tween-80, 1 mM EDTA	50 mM Tris pH 10, 12.5 mM cysteine, 2mM EDTA	19.7	35.1
2B	25 mM GSSG, 6 mM DTT, 0.05% Tween-80, 1 mM EDTA	50 mM Tris pH 8, 12.5 mM cysteine, 2 mM EDTA	24	18

2C	25 mM GSSG, 6 mM DTT, 0.05% Tween-80, 1 mM EDTA	50 mM Tris pH 10, 2.7 mM GSSG, 0.8 mM EDTA	11.9	16
2D	25 mM GSSG, 6 mM DTT, 0.05% Tween-80, 1 mM EDTA	50 mM Tris pH 8, 2.7 mM GSSG, 0.8 mM EDTA	28.8	7.5
3A	6 mM DTT, 0.05% Tween-80, 1 mM EDTA	50 mM Tris pH 10, 12.5 mM cysteine, 2 mM EDTA	44.7	81.3
3B	6 mM DTT, 0.05% Tween-80, 1 mM EDTA	50 mM Tris pH 8, 12.5 mM cysteine, 2 mM EDTA	92.5	96.3
3C	6 mM DTT, 0.05% Tween-80, 1 mM EDTA	50 mM Tris pH 10, 2.7 mM GSSG, 0.8 mM EDTA	95.4	98.9
3D	6 mM DTT, 0.05% Tween-80, 1 mM EDTA	50 mM Tris pH 8, 2.7 mM GSSG, 0.8 mM EDTA	32.7	49.9

Results from Table 7 indicated that refolding conditions 1C, 3B, and 3C (see above table) yielded IL-13Rα2-Fc-B9$_{563}$ fusion protein preparations with roughly equivalent activities, as measured in the TF-1 assay. Condition 3C was chosen as the standard condition with which to produce IL-13Rα2-Fc-B9$_{563}$ fusion protein for the subsequent experiments.

C. Competition assay, IL-13RαR2-Fc-B9 v. IL-13.

In the following experiment, the ability of IL-13Rα2-Fc-B9 fusion protein to inhibit the biological activity of canine IL-13 was determined by the TF-1 cell proliferation assay as described in Example 3B. IL-13 and IL-13RαR2-Fc-B9 were described in Example 3B such that the concentration of IL-13 in the mixture was 50 ng/ml and that of Il-13Rα2-Fc-B9 was as indicated in Table 8.

Table 8. Competition assay, IL-13Rα2-Fc-B9 v. IL-13.

Sample Number	μg/ml IL-13Rα2-Fc-B9	Percent inhibition of IL-13 stimulated TF-1 proliferation
1	13.2	100
2	6.6	99
3	3.3	98
4	1.6	92
5	0.8	38
6	0.4	0
7	0.2	0
8	0.1	0
9	media	0
10	no cells	0

Results indicate that IL-13Rα2-Fc-B9 inhibited the biological activity of canine IL-13 in a dose dependent manner as measured by TF-1 cell proliferation. IL-13Rα2-Fc-B9 at 3.3 and 6.6 μg/ml inhibited over 95% of canine IL-13 activity in the bioassay, and at 1.6 μg/ml inhibited 92% of canine IL-13 activity in the bioassay, indicating the ability of IL-13Rα2-Fc-B9 to bind canine IL-13.

Example 5

This example describes the ability of IL13-Rα2 -Fc-B9 to inhibit IL-13 stimulation of canine peripheral blood mononuclear cells to produce antigen-specific antibody.

A. Preparation of canine PBMC cultures.

Canine peripheral blood mononuclear cells (PBMCs) from flea saliva-sensitized dogs were prepared as described in U.S. Patent No. 5,646,115, issued 7/8/1997, entitled "NOVEL ECTOPARASITE SALIVA PROTEINS AND APPARATUS TO COLLECT SUCH PROTEINS" by Frank et al. incorporated in its entirety herein by reference.

B. Inhibition Assay

PBMCs were cultured for 2 weeks in RPMI media containing 5% normal dog

serum (available from Gemini, Calabasas, CA), 200 mM L-glutamine, 50 µg/ml gentamicin, 1X non-essential amino acids, 1X amino acids, 1X sodium pyruvate, and approximately 5.5 X10-5 M beta-mercaptoethanol. (All these components available from Sigma Chemical Co).

The following sample reactions were set up: (1) medium only plus cells (2) flea saliva product 1 µg/ml(prepared as described in Example 4A); (3) flea saliva product (obtained as described in Example 4A) plus IL-13Rα2-B9 at 6.6 µg/ml; (4) IL-13 (as described in Example 3B) at 50 ng/ml; (5) IL-13 (50 ng/ml) plus IL-13Rα2-B9(6.6 µg/ml), preincubated together before addition to the assay overnight at 4°C; and (6) medium alone, no cells.

The results are set forth in Table 9, below. Flea saliva product (prepared as described in U.S. Patent No. 5,646,115.) was diluted in 100 µl CBC buffer (50 mM sodium carbonate, 50 nM sodium bicarbonate, pH 9.6) and coated at a concentration of 100 nanograms per well onto Immulon II microtiter plates (available from Dynex Technologies, Chantilly, VA). The coated plates were covered and incubated overnight coated at 4°C. Excess fluid was removed and wells were blocked with 200 µl Assay Buffer for one hour at room temperature. Assay buffer contains 4% fetal calf serum (available from Summit Laboratories, Fort Collins, CO) in phosphate buffered saline (PBS, recipe in Sambrook, ibid.), plus 0.05% Tween-20 (available from Sigma, St. Louis, MO). Plates were then washed for 4 cycles on an automatic plate washer (Ultrawash, available from Dynatech Laboratories) using PBS with 0.05% Tween-20.

Samples as described in above, were diluted 1.6 fold with assay buffer and 100µl/well was applied to duplicate wells for an 18 hour incubation at 4°C. Positive control wells received 100µl of a pool of flea allergic dog sera (FAD Pool #9) titered 1:10 to 1:320 by two-fold dilution in the IgE assay and 1:50 to 1:1600 by two fold dilution in the IgG assay (not shown). Control wells, cells from sample (6) were treated identically except that no serum was added. Plates were then washed as described above.

To determine IgE production in the samples, biotinylated human IgE receptor alpha chain ($Fc_{\varepsilon\alpha}R1\alpha$-biotin) (100 µl of 13 ng/ml) prepared as described in US 5, 945,294, ibid., was added and incubated for one hour at room temperature. After this incubation, plates were washed as described above. Streptavidin-horseradish peroxidase

conjugate (0.5 mg/ml, available from KPL Labs, Gaithersburg, MD) was added at a 1:5000 dilution (100 ng/ml) in Assay Buffer for one hour at room temperature, after which plates were washed as described above.

To determine IgG production in the samples, 100 ng of horseradish peroxidase-labeled goat anti-dog IgG (KPL Labs) was added to each well and incubated for a one hour at room temperature. TMB peroxidase substrate system, (2 part system available from KPL Labs, #0-76-00), added at 100 µl per well, was used according to the manufacturer's directions. The color reaction was allowed to proceed until good color development was reached (about 10-15 min); then the reaction was stopped with 100 µl per well of 1 M phosphoric acid.

Bound IgE or IgG was determined by measuring absorbance (Optical Density, or OD) at 450 nm (nanometers) using an ELISA plate reader (such as Spectramax", Molecular Devices, Sunnyvale, CA). Background OD readings in the control wells were subtracted from all numbers. Results are reported in Table 9 as OD multiplied by 1000, and are the mean of duplicate wells. OD numbers greater than 100 are considered to be positive for a reaction with IgE or IgG.

Table 9. Effect of IL-13Rα2-Fc-B9 on Flea-allergic canine PBMC cultures, antigen-specific and IgE and IgG production.

Sample	Sample Treatment	OD, IgE	OD, IgG
1	Medium plus cells	102	1095
2	Flea saliva, 1 µg/ml	42	1397
3	FS + PCaIL13-Rα2 -Fc-B9	43	950
4	IL-13 alone	879	1589
5	IL-13 + PCaIL13-Rα2 -Fc-B9	86	775
6	background (3 wells)	70	881

Results in Table 9 indicate that while IL-13 stimulates antigen-specific IgE production from PBMC, the IL-13Rα2-Fc-B9 chimera prevents the IL-13 stimulation, demonstrating that IL-13Rα2-Fc-B9 chimera binds to IL-13. In samples 2 and 3, the PBMCs were cultured with flea saliva antigen or with flea saliva antigen plus IL-13Rα2-Fc-B9 chimera. However, no effect was seen on the IgE production of the PBMCs in

response to incubation with flea saliva.

While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth in the following claims.